JOURNAL OF AGRICULTURAL RESEARCH

VOL. 46 WASHINGTON, D. C., MAY 15, 1933 No. 10

COMPARATIVE RESPONSES OF A SPRING AND A WINTER WHEAT TO DAY LENGTH AND TEMPERATURE[1]

By ANNIE M. HURD-KARRER [2]

Associate Physiologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Garner and Allard (*15*) [3] have emphasized the fact that in certain cereals a fundamental physiological distinction between spring and winter varieties is shown by the difference in their response to the length of the daily period of illumination. Many other investigators (*5, 8, 10, 11, 12, 19, 26, 37, 39*) have observed such varietal differences, and their reports have called attention to the importance of the day-length response in the geographic distribution of the different varieties of cereals, as especially emphasized in the case of wheat by Wanser (*39*), Doroshenko (*10, 11*), and Tincker (*37*).

The present paper contains observations and measurements illustrating the differences in growth habit, rate of development, height, tillering, and yield of individual wheat plants of two varieties, Hard Federation, an extreme spring type, and Turkey, an extreme winter type, grown in the greenhouse under controlled day-length and temperature conditions.

REVIEW OF LITERATURE

Reviews of the literature on photoperiodism in plants by Maximov (*25*), Kellerman (*21*), Redington (*34*), and Berkley (*7*) will serve to give the background of the present investigation.

As with most of the plants studied, the major emphasis in the case of wheat has been on the acceleration of reproductive processes by long light periods, and their retardation by short ones (*2, 3, 4, 5, 9, 10, 11, 12, 15, 22, 23, 26, 36, 37*). The extent to which the vegetative period can be shortened by increasing the day length differs for different varieties, the spring wheats having been found especially responsive to the hastening influence of a long light period. These investigations leave no doubt that the day-length responses of spring and winter types of cereals are as distinctive as their temperature adaptations (*12, 16, 26, 29*).

The length of the daily period of illumination has been found to determine not only height, tillering, and date of heading, but also root development (*15, 26, 40*), leaf and head development (*10, 11*), and

[1] Received for publication Feb. 21, 1933; issued June, 1933.

[2] The writer is indebted to John W. Taylor, of the Division of Cereal Crops and Diseases, and to H. A. Allard, of the Division of Tobacco and Plant Nutrition, for their helpful suggestions in the course of this investigation; to A. G. Johnson and R. W. Leukel, of the Division of Cereal Crops and Diseases, for planning and supervising the installation of the temperature-control equipment; and to Fred Young for designing equipment used in controlling day length.

[3] Reference is made by number (italic) to Literature Cited, p. 886.

growth habit (*19*). In general it can be concluded from the literature that as the day length is made shorter the plants are more vegetative, with more tillers and larger leaves. As it is made longer, opposite effects are obtained. Some of Adams' results (*1, 3*) apparently constitute exceptions to these generalizations, but the explanation probably lies in his experimental procedure.

The interrelationship of temperature, light intensity, soil moisture, and other environmental factors in these various day-length effects has been discussed by many investigators (*2, 4, 6, 12, 14, 15, 17, 18, 19, 26*). Their reports confirm those of Garner and Allard in giving evidence that other factors may modify, to a certain extent, responses to the length of the daily light period.

METHODS

On December 2, 1930, seeds of a spring-wheat variety, Hard Federation (C. I.[4] No. 4733), and of a winter variety, Turkey (C. I. No. 1558), were sown in greenhouse benches at the Arlington Experiment Farm, Rosslyn, Va., in soil that was 10 inches deep. The plants were 4 inches apart in rows 6 inches apart. Different day-length conditions were provided for the different benches by means of artificial light and light-tight covers. The sowings were duplicated in the south-end sections of two adjacent greenhouses, one of which was kept at a favorable low temperature (12° ± 1° C.), the other at a temperature too high for the best growth of wheat (21° ± 1°). The temperature was regulated by means of thermostatic control of the steam valves and of the dampers admitting cold air from the outside. An air distributor, 6 feet above the floor, circulated the incoming cold air fairly uniformly, so that the total variation in temperature in those parts of the houses used for the experiment was not over 1 degree.

Combination soil and air thermographs and standardized maximum and minimum thermometers were kept on stands 1 foot above the soil in each bench. During the period of temperature control—from December 2, 1930, the date of sowing, until April 3, 1931, when outdoor temperatures became too high—the cold house was held at 12° ± 1° C. except for a few hours on sunny afternoons, when it often rose to 15° in midafternoon, rarely to 20°. In the warm house the temperature was similarly well controlled at 21° ± 1° until April 8. On sunny afternoons it rose to 25°, but rarely above. Soil temperatures were approximately 2 degrees lower than the air temperatures in each house. Unless otherwise specified, the temperatures referred to in this paper are air temperatures.

Although the desired temperatures could not be maintained in either house after the first week of April, the low-temperature house was kept approximately 5 degrees colder than the other by means of a sprinkler system which kept a thin sheet of water running over practically the entire area of the glass of the roof and sides.[5]

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

[5] This method of lowering the temperature in the greenhouse, suggested by S. Karrer, was inexpensive and easily installed. Water pipes with spray nozzles at 1-foot intervals were laid on the roof just below the ventilators, and across the south end of the house. The nozzles were directed downward at such an angle as to cause the water to strike the glass a short distance from the pipe, where it spread to the width of the pane.

[6] Throughout the following discussions the terms "long-day," "short-day," "low-temperature," and "high-temperature" are used to denote the relative conditions under which the plants were grown, and not, in the sense of Garner and Allard (*14*), to denote the optimum condition for flowering. In the latter sense wheat is a long-day plant because flowering is induced by long days, and is delayed, as in Hard Federation, or prevented, as may occur in Turkey, by short days.

For the long-day [6] plants in each house the natural day was supplemented with light from six 100-watt Mazda C lamps in standard deep-bowl reflectors, distributed at 32-inch intervals, 4 feet above the bench. These lights were turned on and off automatically by a time switch which was adjusted periodically so as to maintain a constant 17-hour light period. The intensity of the artificial light at the level of the bench varied but slightly from 40 foot-candles. As the plants increased in height, the distance of the lights from the bench was increased to 5 feet. The thermograph records showed no discernible heating effects of these lights at the level of the plants.

The short-day condition was produced for the plants in another bench in each house by means of a light-tight black cloth cover supported on a slender framework 4 feet above the level of the bench. The cover was hung several inches out from the bench in order to insure ventilation. At the corners the side and end pieces overlapped and were attached to the framework with snap fasteners when the cover was in place. A piece of 1-inch pipe constituted the back edge of the framework, and to it was attached a crank handle by means of which the curtain could be rolled upon the pipe and lifted to one side when not in place over the bench. The plants were darkened every day from 4 p. m. until 8 a. m., leaving them a light period of eight hours in the middle of the day. The cover was thick enough to protect the plants from the lights of the neighboring long-day bench, and was always in place before these lights were turned on.

Control plants with the natural light period were grown on an adjacent bench and protected from the lights over the long-day bench by a heavy black curtain 6 feet high, hung in the aisle and extending well past the ends of the benches. This curtain was in place whenever the lights were on, and was pulled to one end, where it did not shade the plants, when the lights were turned off. The natural length of day, from sunrise to sunset, increased over the period of the experiments from 9.5 hours in December to 15 hours in June.

The following observations and measurements are based on approximately 60 plants of each variety, exclusive of border plants, grown under each of these six different conditions of day length and temperature.

RESULTS

EFFECTS OF DAY LENGTH AND TEMPERATURE ON RATE AND TYPE OF DEVELOPMENT

The seedlings of the low-temperature house (12° ± 1° C.) emerged in 10 days, and those of the high-temperature house (21° ± 1°) in 6 days. The long (17-hour) light period so accelerated the development of the young plants of the spring variety, Hard Federation, that at first they greatly exceeded the others in height. At the natural and at the short (8-hour) days, Turkey, the winter variety, assumed, for about three months, the recumbent growth habit, typical of young plants of this and similar varieties in winter; but at the 17-hour day the plants were erect or semierect from the first, as

in experiments previously reported by the writer (*19*).[7] In the low-temperature house these erect plants matured normally and produced good yields of grain. In the high-temperature house they remained largely vegetative, as did the Turkey plants of the other light periods at this temperature.

The contrast between the erect growth of the young plants of Turkey at the long day and their recumbent growth at the shorter ones, especially marked at the low temperature, is illustrated in Figure 1, which also shows the relative development of Hard Federation plants of the same age. The plants of Turkey with the 8-hour day, A, were still prostrate at the time the photographs were taken, while the control plants, B, with the natural day, which was nearly 11 hours long at this time, had become semierect, indicating the resumption of active growth. The 17-hour-day plants, C, were never prostrate.

The comparative rates of development of Hard Federation and Turkey under the different conditions are shown in Table 1, where the dates of first flowering, together with the averaged growth measurements and their probable errors, are recorded. Heading occurred during the week or 10 days preceding the date of first flowering, except in the case of the short-day plants, which headed irregularly over a longer period.

Representative plants from the different environments, selected for photographing when maximum growth had been attained but while most of the foliage was still green, are shown on the same scale in Figures 2 and 3. Later, after the plants had matured and their measurements were obtained and averaged, the photographs of plants conforming most closely to the averages for the groups were chosen for the illustrations. Occasionally none of the plants photographed from a given group happened to conform to the size shown by the averages in Table 1 to be typical. Such was the case in Figure 3, where all the plants have more than the average number of tillers for their group.

[7] Since the present paper was prepared, Forster, Tincker, Vasey, and Wadham (*13*) have reported that in their wheats growth did not completely cease at temperatures as high as 10° C., and that therefore they do not agree with the writer's statement (*19, p. 119*) that a resting stage is produced by 8 and 9.5-hour light periods at this temperature. The term "resting stage" was used in this earlier publication to designate the prostrate stage of development of Turkey in winter, preceding the erect leafy growth of the later period of the tillering stage. In Turkey and similar varieties the plants are recumbent during this early tillering stage, as described and illustrated by Percival (*31, p. 69–71*), and growth is almost imperceptible over a considerable period. A rest period is referred to by Maximov (*28, p. 290*) and others as typical of some cereals in winter, "* * * when growth completely ceases and the plant enters into a state of rest." Maximov states further: "This condition may be of various intensity and duration. It may involve the whole plant or only parts of it." Undoubtedly, as Forster and his associates have stated, all growth during the recumbent stage is not completely stopped in winter cereals at a temperature as high as 10° to 12°, the low temperature of this and the previous investigation (*19*). However, whether or not there is a true dormant or rest period is immaterial to the purpose of these investigations, the term being used with the sole purpose of designating the period during which the plant is in the prostrate or recumbent condition characteristic of the early tillering stage of Turkey and similar winter wheats grown with short light periods.

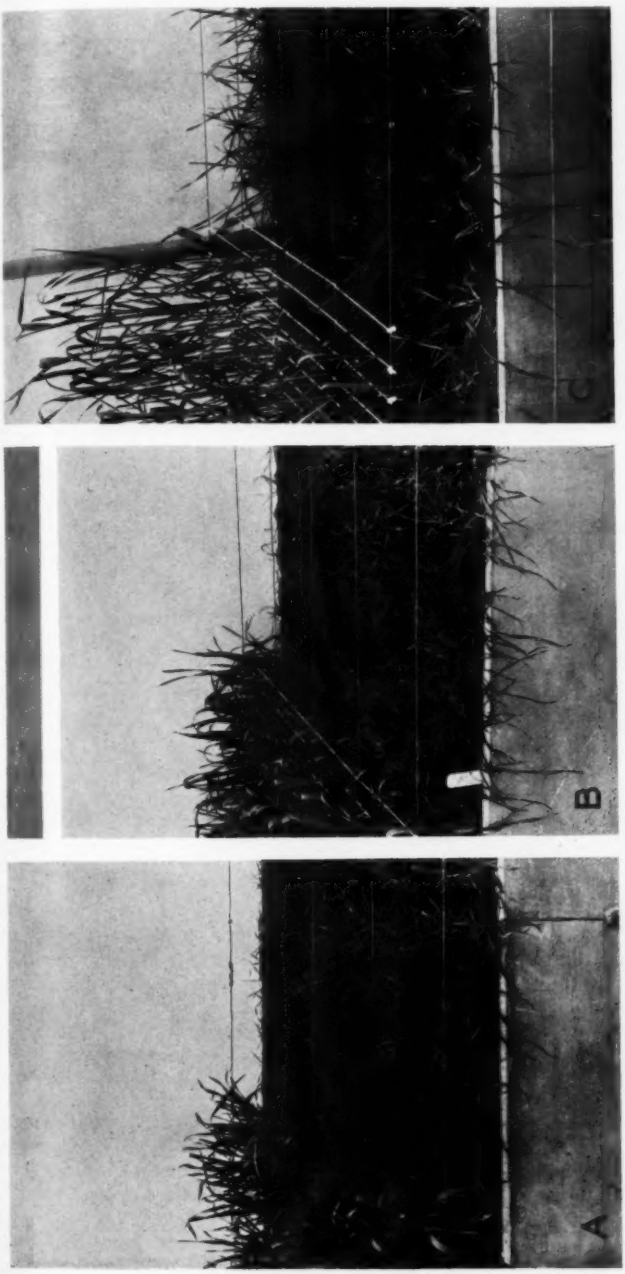

FIGURE 1.—Comparative development of winter-sown Turkey wheat (at right in each photograph) and Hard Federation (at left) 10 weeks after sowing, grown with constant low temperature (12°±1° C.) and different light periods: A, Short day (8 hours); B, natural day (9.5 to 10.5 hours); C, long day (17 hours)

TABLE 1.—*Effects of day length and temperature on the average height, tillering, head development, and yield of a spring wheat (Hard Federation) and a winter wheat (Turkey) sown December 2, 1930*

Item	Short day (8 hours)	Natural day (9.5–15 hours)	Long day (17 hours)
	Hard Federation at 12°±1° C.		
Date of first flowering	May 9	Apr. 13	Feb. 19
Dry weight of straw, grams	9.5	7.7	1.2
Height of plants, centimeters	90±0.75	98±0.93	66±0.86
Total tillers per plant, number	5.8±.27	3.7±.16	[a] 3.8±.16
Fertile heads per plant, do	3.9±.19	3.5±.17	1.3±.10
Length of heads, centimeters	9.5±.07	8.6±.06	5.4±.05
Kernel weight, gram	.035±.0007	.054±.0005	.055±.0009
Weight of grain per fertile head, do	.51±.02	.85±.06	.37±.02
Yield of grain per plant, do	1.91±.11	2.81±.21	.45±.05
	Hard Federation at 21°±1° C.		
Date of first flowering	Apr. 25	Mar. 18	Jan. 13
Dry weight of straw, grams	10.3	8.1	[b]
Height of plants, centimeters	82±1.20	84±1.07	48±.77
Total tillers per plant, number	8.1±.32	6.2±.28	2.7±.11
Fertile heads per plant, do	2.9±.14	4.1±.25	1.6±.06
Length of heads, centimeters	8.5±.08	7.8±.05	5.4±.06
Kernel weight, gram	.028±.0004	.039±.0007	.036±.0007
Weight of grain per fertile head, do	.24±.04	.61±.03	.38±.02
Yield of grain per plant, do	.77±.06	2.40±.18	.67±.03
	Turkey at 12°±1° C.		
Date of first flowering	June 8	May 16	Apr. 13
Dry weight of straw, grams	12.9	8.7	3.8
Height of plants, centimeters	110±0.76	115±0.88	101±0.71
Total tillers per plant, number	6.6±.28	5.3±.24	3.4±.09
Fertile heads per plant, do	2.0±.13	4.3±.17	2.9±.09
Length of heads, centimeters	13.7±.11	10.0±.05	8.4±.04
Kernel weight, gram	.022±.0008	.035±.0002	.043±.0005
Weight of grain per fertile head, do	.23±.02	.98±.04	1.15±.03
Yield of grain per plant, do	[c] .53±.04	4.06±.19	3.57±.09

[a] Very short secondary tillers without grain and but a few inches long, characteristic of these plants, were not included in this average. If included, the average would be 6.9.

[b] Straw inadvertently discarded.

[c] In the year preceding and the year following, in both of which the seed was sown 2 weeks later than in this experiment, the heads of Turkey at the short day produced no grain. (Table 2.)

Hard Federation was extremely sensitive to the forcing action of the lengthened light period. At both temperatures the plants with the 17-hour day were forced into the reproductive stage so rapidly that there was insufficient leaf development before maturation processes began. The plants were extremely stunted, with short, narrow leaves and small, poorly developed heads. (Fig. 2, C, F.) In the low-temperature house they began to flower the third week of February, 10 weeks after emergence. They were eight weeks ahead of the control plants (fig. 2, B), which flowered the second week of April. In the high-temperature house, development was still more rapid, the long-day plants flowering the second week of January, only five weeks from emergence and nine weeks ahead of the control plants (fig. 2, E), which bloomed the third week of March.

Maturation of the short-day plants of Hard Federation was as markedly retarded as that of the long-day plants was hastened. Whereas the rapid development at the 17-hour day had resulted in stunted plants with reduced foliage, the 8-hour day caused a highly vegetative type of growth, with increased tillering and large leaves. (Fig. 2, A, D.) In the cold house the plants began to flower the second week of May, a month later than the control plants with the natural light period. At the higher temperature they flowered the last week of April, more than five weeks later than the natural-day plants in that house.

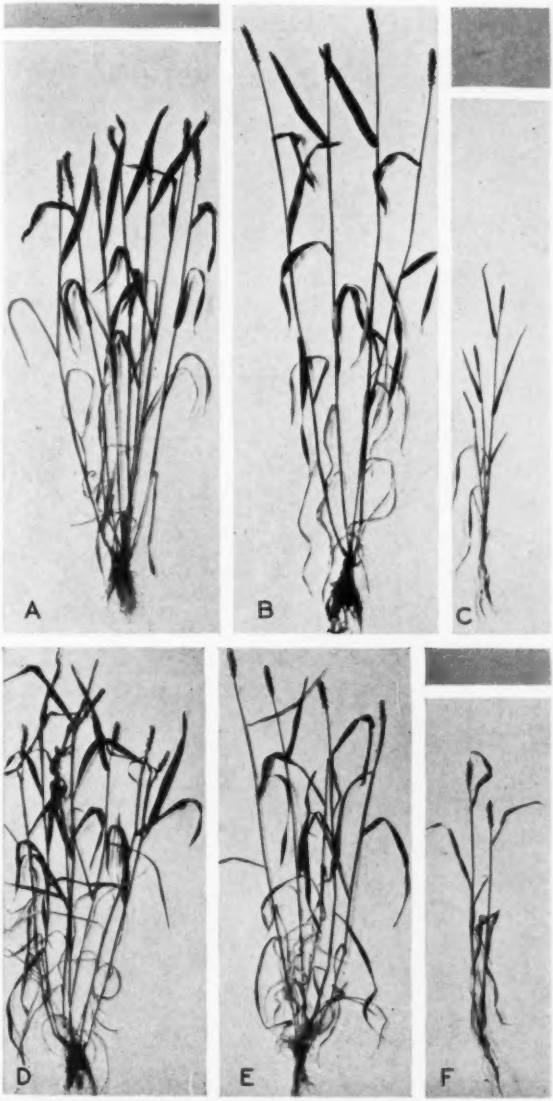

FIGURE 2.—Mature plants of winter-sown Hard Federation wheat grown with different light periods and temperatures (12±1° and 21±1° C. for the first four months): A, B, C, Short-day, natural-day, and long-day plants, respectively, grown at the lower temperature; D, E, F, short-day, natural-day, and long-day plants, respectively, grown at the higher temperature

At the low temperature, Turkey, the winter variety, while less susceptible to the forcing action of the lengthened light period than Hard Federation, also developed more rapidly with the 17-hour day than with the natural day. The plants were not stunted as were the

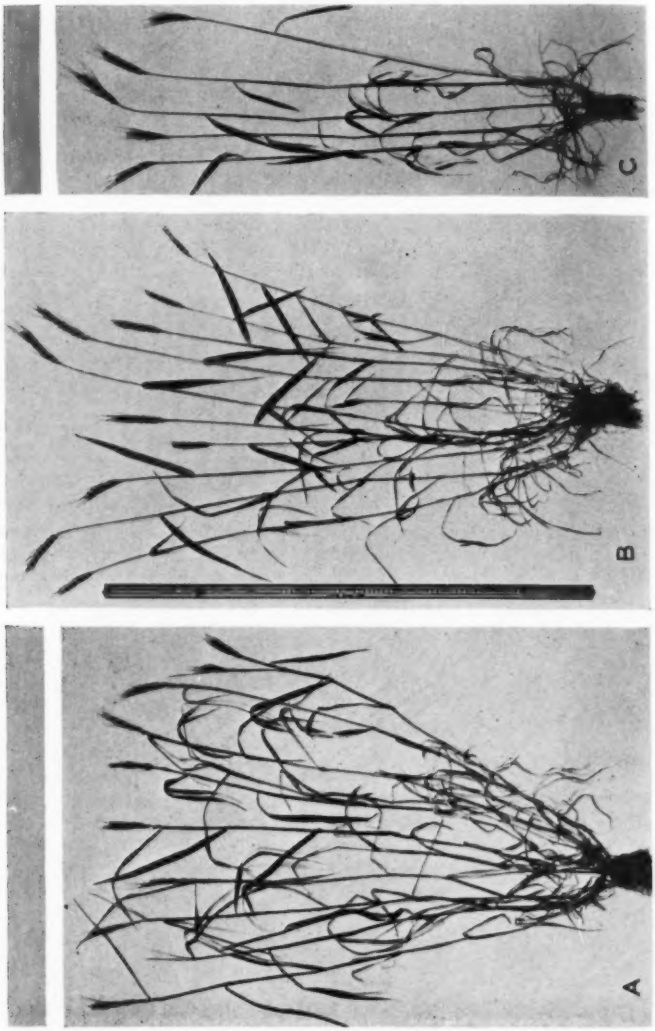

FIGURE 3.—Mature plants of winter-sown Turkey wheat grown with different light periods and, for the first four months, at a constant temperature of 12±1° C.: A, Short day (8 hours); B, natural day (0.5 to 15 hours); C, long day (17 hours)

long-day plants of the more rapidly developing spring variety, but on the contrary compared favorably, except for their reduced tillering, with the natural-day controls. (Fig. 3, B, C.) Flowering occurred during the second week of April, five weeks ahead of the natural-day plants, while those of Hard Federation growing beside them had

flowered the third week of February, eight weeks ahead of their controls. The short day resulted in a vegetative type of growth, as in Hard Federation, with maturation considerably delayed. (Fig. 3, A.) Thus, at the 8-hour day Turkey did not head until June 8, three weeks later than the natural-day plants.

In the high-temperature house but few heads were produced by Turkey at any day length, most of the plants being completely sterile. They remained green and mostly vegetative until the following October, when they were discarded. Excessive tillering, together with the common failure of the culms to elongate, gave the plants of all the light periods the bushy appearance characteristic of spring-sown winter wheats that fail to head (*6*). The photographs in Figure 4 were taken in August, when the plants were still green, long after all the other lots had ripened. The vegetative condition of the long-day plants in this house gave evidence of the fact that the ordinarily dominant action of the light period becomes imperceptible at temperatures unfavorable for reproduction.

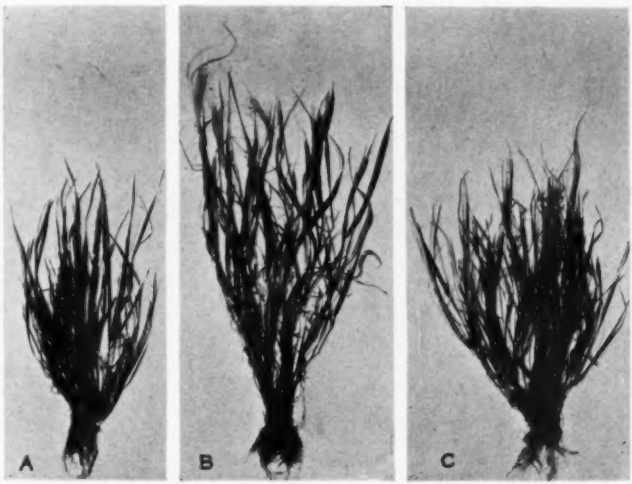

FIGURE 4.—Effect of the higher temperature (21°±1° C. for first four months) on the growth of Turkey at the different day lengths: A, Short day (8 hours); B, natural day (9.5 to 15 hours); C, long day (17 hours)

The tendency of the shortened day to favor continued vegetative growth and that of the long day to inhibit it are evident in both the winter and the spring variety on comparing the weights of the dried plants from the different environments. The average weights of straw per plant shown in Table 1 were obtained by weighing the fully ripened air-dry plants, exclusive of the heads and roots. In the low-temperature house the average weight of a short-day plant of Turkey was more than three times that of a long-day plant. In the case of corresponding plants of Hard Federation, the short-day plants averaged eight times the weight of those with the long day. The straw of the long-day plants of Hard Federation from the high-temperature house was inadvertently discarded, but approximately the same relation is evident from the appearance of the plants in

Figure 2, F. The weights of the natural-day plants of both varieties were intermediate between those of the short and long days, but re-

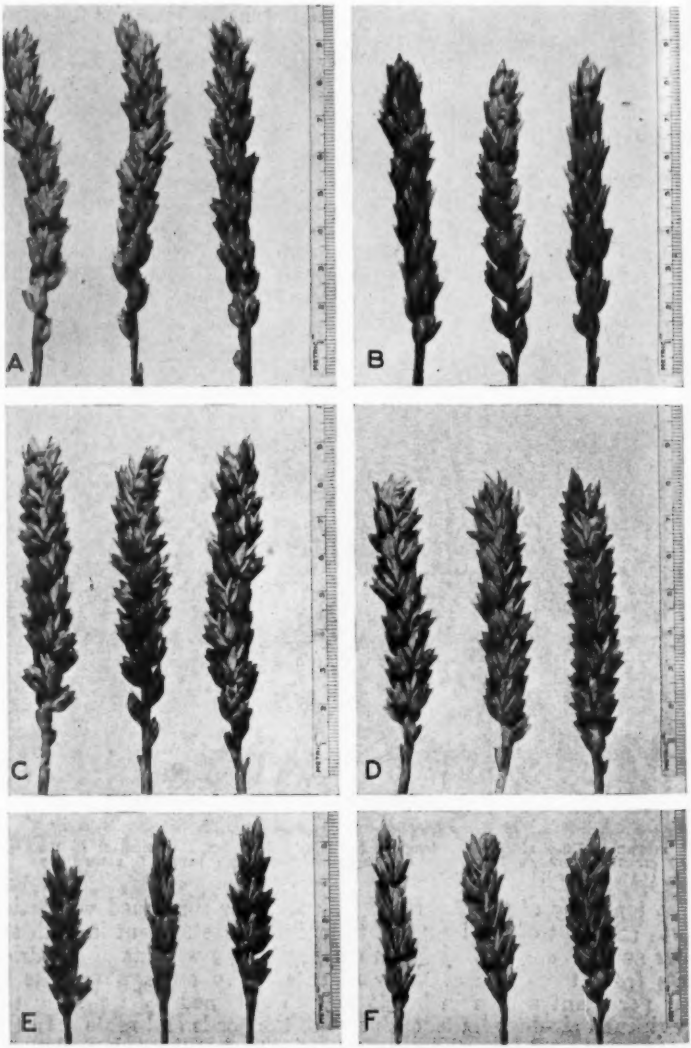

FIGURE 5.—Typical heads of Hard Federation wheat grown with different day lengths and temperatures (12° and 21° C. for the first four months): A and B, Short day (8 hours) at the low and high temperatures, respectively; C and D, natural day (9.5 to 15 hours), at the low and high temperatures, respectively; E and F, long day (17 hours) at the low and high temperatures, respectively

duction in size by the long day was always more pronounced than was the increase brought about by the short day.

The average heights recorded in Table 1 represent the final measurements on the mature plants, including the heads. The data show that the 17-hour light period shortened the plants in every case, the effect on Hard Federation being much more extreme than the effect on Turkey. The 8-hour day also reduced the average height of the plants of both varieties, but the effect was less pronounced than that of the lengthened light period. At every day length the plants of the cold house were taller than the corresponding plants of the warm house, the difference reflecting the injurious effects of temperatures above 20° C. on the growth of even the spring wheat.

The number of tillers per plant was highest in both varieties at the short day, and generally lowest at the long day. If only normal tillers with heads were counted, the number was always lowest at the long day. At equivalent day lengths, more tillers were produced at the high than at the low temperature, except in Hard Federation at the long day.

FIGURE 6.—Typical heads of Turkey grown at 12°±1° C. for the first four months, with different light periods: A, Short day (8 hours); B, natural day (9.5 to 15 hours); C, long day (17 hours)

Among the conspicuous formative effects of the length of the light period were the effects on head length. (Table 1.) In both Hard Federation (fig. 5) and Turkey (fig. 6) the long day produced abnormally short heads. The short day produced very long heads, largely as the result of abnormal elongation of the basal internodes of the rachis. This elongation was especially pronounced in Turkey, some of the heads reaching 20 cm, measured from the lowest node of the rachis. Occasional abnormal heads like those in Figure 7, showing indeterminate growth of the spikelets, were produced by Turkey at this day length.

Other structural abnormalities were the shortened peduncles of the plants of the short day. (Figs. 2, A, D; 3, A.) Sometimes elongation of the peduncle ceased as soon as the head emerged from the boot.

The short day resulted also in a tendency toward abnormal branching. Sometimes the culm branched at one of the upper nodes (fig. 3, A) and sometimes nearer the base. This effect calls to mind Garner and Allard's finding (*15*) that a change in the light period from optimum to suboptimum for stem elongation promotes branching.

A striking abnormality, occurring frequently in the long-day plants of Turkey at the high temperature, is shown in Figure 8. In these plants, heading was almost completely inhibited, and masses of leaves and roots often grew from the nodes of the culm. Although most pronounced at the 17-hour day, occasional instances occurred at the natural day, which was 15 hours long in July when the tendency was first observed.

FIGURE 7.—Abnormal heads of Turkey grown at the low temperature with a short light period (8 hours)

EFFECTS OF DAY LENGTH AND TEMPERATURE ON PRODUCTION OF GRAIN

As a result of a tendency toward increased sterility in both varieties with light periods other than the natural one, the relationship of day length to yield was not that which might have been expected from its effects on tillering. In Hard Federation the abnormally long heads of the short-day plants, as well as the short stunted heads of the long-day plants, were poorly developed. Maximum yield per plant, therefore, occurred with the natural length of day and not in the many-tillered short-day plants. In Turkey also the poorly developed heads of the short-day plants and the reduced tillering of the long-day plants resulted in the greatest yield occurring at the natural day. (Table 1.)

In the case of the winter variety, Turkey, the shortened light period was more damaging to yield than the long one, as many heads were completely sterile or only partly filled with small and shriveled grains. But in the case of the spring variety, Hard Federation, the long light period was the more damaging, many heads being sterile or incompletely filled.

An interesting distinction between the two varieties appeared also in the relative effects of the lengthened light period on individual head yield. Table 1 shows that in the low-temperature house Turkey

produced the highest yields of grain per head with the 17-hour day, but in Hard Federation the highest yield occurred at the natural day, the long day greatly reducing the number of grains per head. This same difference appeared also in the other experiments. (Table 2.)

The short-day plants of both varieties produced the smallest grains. (Table 1.) Those of Turkey were especially poor, being shriveled, dark in color, and much reduced in weight. Thus the short-day con-

FIGURE 8.—Abnormal growth of long-day Turkey wheat plants grown at the high temperature. Bunches of leaves and roots grew from the nodes, the plants remaining erect

dition reduced yields not only by increasing sterility but by reducing the size of such grains as were formed. In other experiments (Table 2), for which the seed was sown two weeks later, the short day prevented flowering in Turkey and so resulted in complete sterility.

The 17-hour day did not affect the kernel weight of Hard Federation appreciably at either temperature, so the greatly reduced grain weights shown in Table 1 for the long-day plants were caused by a decrease in the number of fertile tillers per plant and grains per head, rather than by a decrease in the size of the kernels. In the winter variety, Turkey, there was an increase in the average kernel weight at the long day. A similar increase occurred in this variety in all the experiments. (Table 2.)

Relative kernel size for the different groups of plants is illustrated in Figure 9. This figure shows the reduced size of the grains of the short-day plants of both varieties, and the large plump grains of the long-day low-temperature plants. The high-temperature grains were always smaller than the corresponding ones from the low-temperature plants.

The following year (1932) grain from each of these seed lots was grown in the low-temperature house with the natural light period.

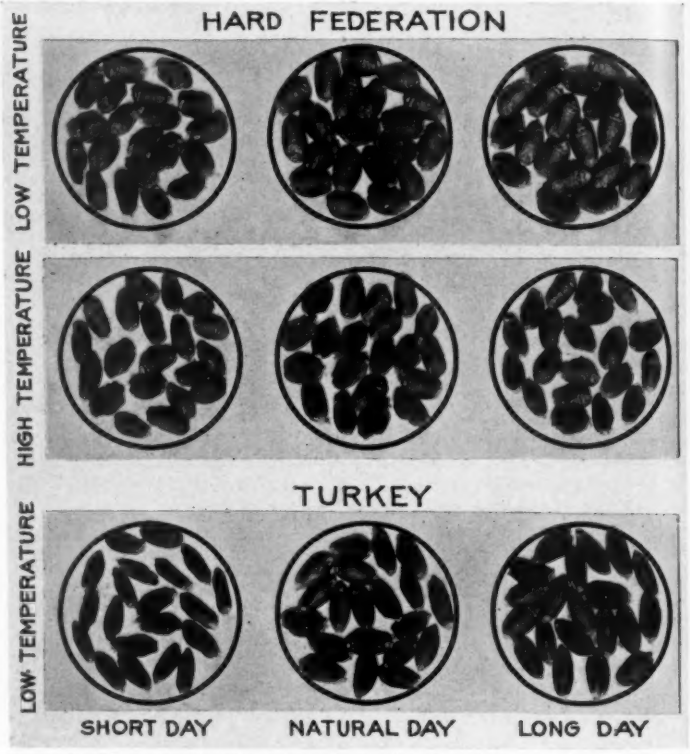

FIGURE 9.—Effects of day length and temperature on kernel development of wheat. (Twenty grains in each circle)

Except for somewhat reduced size in early growth stages, the plants grown from the small shriveled grains of the short day were very similar to those from the larger grains of the other day lengths. There were no significant differences in height, tillering, dry weight, or yield of any of the plants. It is concluded, therefore, that the conditions of day length and temperature under which the grain was produced had no appreciable effect on the development of the plants grown from the grain.

SUPPLEMENTARY EXPERIMENTS

The question arises in connection with the generalizations discussed in the preceding sections as to the reproducibility of these effects of day length and temperature. Data from several similar experiments but with different dates of sowing and different spacings are summarized in Table 2 in order to show that the relative effects of the different environments and the varietal differences in the responses to these environments were not fortuitous variations.

The experiment with spring-sown grain (A) included only Hard Federation, and differed from that just described in its closer spacing, the plants being 2 instead of 4 inches apart. The experiment with grain sown in the winter of 1931 (B) also differed with respect to spacing, in that the rows were 1 foot apart instead of 6 inches. The experiment with grain sown in the winter of 1929 (C) resembled that described in the preceding sections of this paper, the plants being spaced at 4-inch intervals in rows 6 inches apart. The experiment A differed from all the rest in that temperature control could be maintained for the first month of growth only, whereas the winter sowings had controlled temperatures for the first four months. The temperatures were similar in all the experiments, namely, between 10° and 14° C. in the low-temperature house and between 20° and 23° in the high-temperature house. All border plants were discarded.

The data in Table 2 are in accordance with the generalizations indicated by the data in Table 1. They show that the 17-hour day accelerated the development of both Hard Federation and Turkey and produced smaller plants, with lower dry weights and reduced tillering. Acceleration of heading was less marked in Turkey than in Hard Federation, as before. Yield per head at the long day was always higher than at the natural day in Turkey but lower in Hard Federation. The short day consistently produced large vegetative plants with the most tillers, whose development was retarded. Grain production was decreased or, in Turkey, entirely prevented by the short day.

A significant difference between the Turkey plants of the experiments of Table 2 and those of Table 1 was the failure of the former to flower at the short day. The difference may be attributed to the fact that the grain for the experiments of Table 2 was sown two weeks later than that of Table 1, the retardation by the short day resulting in the later-sown lots reaching the preflowering stage when greenhouse temperatures were higher.

TABLE 2.—*Effects of day length and temperature on average height, tillering, head development, and yield of Hard Federation and Turkey wheat in supplementary experiments, 1930–1932*

Item	Experiment [a]	Hard Federation						Turkey		
		Low temperature			High temperature			Low temperature		
		Short day (8 hours)	Natural day (9.5–15 hours)	Long day (17 hours)	Short day (8 hours)	Natural day (9.5–15 hours)	Long day (17 hours)	Short day (8 hours)	Natural day (9.5–15 hours)	Long day (17 hours)
Date of first flowering	A	June 13	May 15	May 7	June 30	May 4	Apr. 18			
	B	May 4	Apr. 17	Feb. 29					May 15	May 4
Dry weight of straw, grams	A	2.9	2.7	1.1	3.8	2.9	0.8	12.7	11.4	8.1
	B	6.3	8.2	1.6						
Height of plants, centimeters	A	72	81	55	72	62	37			
	B	76	99	64				76	118	119
Total tillers per plant, number	A	1.8	1.3	1.1	3.9	1.5	1.4	12.8	9.3	7.2
	B	5.9	5.0	3.9						
Fertile heads per plant, do	A	1.1	1.1	1.0	1.5	1.4	1.0	0	8.6	7.0
	B	4.4	4.4	1.1						
Length of heads, centimeters	A	9.0	7.2	4.6	7.8	7.2	4.3			
	C	8.9	8.1	4.6	8.0	7.6	3.9			
Kernel weight, gram	A	.030	.045	.045	.03	.041	.033			
	B	.021	.049	.048				0	.032	.037
	C	.034	.049	.045	.03	.041	.032	0	.038	.043
Weight of grain per fertile head, do	A	.23	1.00	.51	.11	.95	.30			
	B	.26	1.44	.33				0	.78	.88
	C	.79	1.17	.03	.40	.88	.21	0	1.04	1.18
Yield of grain per plant, do	A	.25	1.10	.51	.16	1.33	31			
	B	1.08	6.23	.38				0	6.89	6.07

[a] Experiment A, seed sown Mar. 2, 1931; B, seed sown Dec. 15, 1931; C, seed sown Dec. 13, 1929.

DISCUSSION

A shortened daily light period induces an abnormally vegetative type of growth in wheat, whereas, at temperatures low enough to permit heading, a lengthened one induces abnormally hastened reproduction. However, the results of the present investigation show that plants so rapidly forced into the reproductive stage by a long light period are not made more fruitful thereby, but, on the contrary, yield is reduced, especially in the case of a spring wheat like Hard Federation. Plants of this variety are stunted, the leaves small, and the heads often sterile. The winter variety, Turkey, is not forced so rapidly into reproduction by a long day, but, at favorably low temperatures, it grows tall and vigorously, its leaves are large, and it produces an even higher yield of grain per head than it does with the natural day. Nevertheless it does not produce the greatest yield per plant at the long day, for the number of heads is reduced.

It is interesting to note that although a shortened light period always increased the number of tillers per plant in both varieties, and, in Turkey, a lengthened one increased the kernel and head weights, still the highest total yield of both varieties was produced by the plants with the natural day, possibly because of the favorable effect of the changing light period (*19*, *27*, *33*), which increased from 9.5 to 15 hours during the experiments.

Differences between the reactions of the spring and the winter variety to the different environments were conspicuous at every stage of development. In the early tillering stage, the prostrate growth habit of the short-day and natural-day plants of Turkey distinguished them from the erect plants of Hard Federation in the same benches. Within the first 5 to 10 weeks after emergence, depending on the temperature, heads were appearing in the long-day plants of Hard Federation, while Turkey in the same bench was still in the tillering stage. Turkey continued in this vegetative stage for some time after the spring plants had fully matured; so, although the long-day plants of Hard Federation were much taller at first than the adjacent plants of the winter variety, their growth was soon ended by the early maturation of the heads, leaving the plants abnormally short and with reduced leaf and head development. At the low temperature the more slowly developing long-day plants of Turkey grew to twice the height of the Hard Federation plants beside them and produced fully developed leaves and heads. The extreme reduction in yield in Hard Federation at the long day, with less injury at the short day, and, conversely, in Turkey, the occurrence of extreme injury at the short day with increased head and kernel weights at the long day, gave interesting evidence of a fundamental distinction between spring and winter wheats. Other evidence of the physiological difference between the two varieties was, of course, the greater tolerance of Hard Federation for the higher temperatures.

The appearance and measurements of the plants in the present investigation show that excellent growth can be obtained in the greenhouse with no source of ultraviolet light. In 1931 the control plants of the winter variety, Turkey, with the natural length of day averaged 115 cm in height at maturity, with an average of 5.3 tillers and 4 g of grain per plant, testing 62 pounds to the bushel. In 1932, when the rows were 1 foot apart instead of 6 inches, still larger plants

were obtained which averaged 8.6 fertile heads, giving 6.9 g of grain per plant. (Table 2.) The plants, therefore, compared favorably with field-grown plants (*6*) of this variety.

The excellent growth and yield of the plants of Turkey at the 17-hour day were of special interest because the recumbent growth habit typical of this variety in a natural winter environment at this latitude was inhibited by the long light period. The subsequent normal development of the plants showed, in agreement with the conclusions of other investigators (*29*, *30*, *38*), that a resting or recumbent stage was not essential for good yield. Since 17-hour-day plants of this variety yielded so well in all the experiments, the suggestion that the failure of spring plantings of winter wheat to head may be attributed to too long light periods (*29*)—or to the resulting absence of a prostrate growth phase in the tillering stage (*19*)—now seems untenable, for the longest summer day is only 15 hours at this latitude. In view of the fact that temperatures above 20° C. in the greenhouse are so deleterious to winter wheat (*20*) and, in the present experiments, always prevented normal development at all the day lengths used, it seems probable that the onset of warm weather before the end of the long vegetative period is responsible for the abnormal growth of Turkey in spring sowings.

Gassner (*16*), McKinney and Sando (*24*), Maximov (*26*), and others have shown that stimulation of the transition from vegetative to reproductive growth in winter wheat can be brought about by germinating the plants at temperatures near freezing. However, Murinov (*30*) and Wacar (*38*) have reported that, in the winter varieties they studied, very low germination temperatures were not essential for heading. It now seems from other investigations (*29*, *32*) that very low temperatures in early stages stimulate heading in the case of late sowings only, for when germination takes place in the winter such low temperatures have no noticeable effect, presumably because the short winter day supplies a similar stimulus (*27*). In accordance with these reports, the low-temperature plants of the present investigation (for all of which the seed was sown in December) progressed normally from the vegetative condition to reproduction with normal yields of grain without experiencing either soil or air temperatures below 9.5° C. It would seem, therefore, that statements (*35*, *p. 53*) to the effect that winter cereals "grown from seed germinated above 5° C. and not allowed during growth to fall below this temperature do not form ears but grow vegetatively only" are not applicable to the winter wheat used in these investigations.

It is of interest to note that three groups of the Hard Federation plants, including one in the low-temperature house (Table 1) flowered within the period of temperature control, which continued through the first week of April. With the exception of the natural-day plants, for which the light period was increasing, these plants flowered with no apparent change in the external environment other than the change in light intensity as the season advanced. This fact would appear to controvert the opinion (*27*) that a rise in temperature as well as an increase in day length is necessary for further development after the plant has reached the preflowering stage.

Wanser (*39*) thought that specific critical day lengths are necessary to bring about the transition from the vegetative to the reproductive

phase of growth in winter wheats, and to initiate jointing and heading. The fact that in the present investigation Turkey plants have progressed from the seedling stage to culm elongation, flowering, and maturation at both the constant short and constant long days shows that the stimulus of a changing length of day is not essential for development in all winter wheats.

Turkey and some other varieties of winter wheats have been shown (*19, 27, 33*) to be stimulated to more rapid development by transferring them from short to long light periods in early stages of growth. In the writer's experiments, transplanting young Turkey plants from the short-day to the long-day bench at the age of 2 months, when they were still in the recumbent stage, resulted in rapid emergence from this stage. Culm elongation proceeded so rapidly that the plants soon surpassed all the others. Flowering and maturation of such transplanted plants in experiments of three different years occurred without exception from one to two weeks earlier than in the adjacent plants with the constant long day, which were themselves two months earlier in heading than were the natural-day plants.

The various reports (*16, 19, 24, 26, 27, 29, 32, 33, 38*) of favorable effects of a change from low temperatures or short days in early growth stages to higher temperatures or to longer days in later ones have proved that under some conditions such changes hasten the subsequent transition from vegetative to reproductive growth. There still remains the question as to what brings about the transitions in growth phases that occur in the absence of any apparent change in the external environment.

SUMMARY

Hard Federation, a typical spring wheat, and Turkey, an extreme winter type, were grown in the greenhouse with short (8 hours), natural (9.5 to 15 hours), and long (17 hours) light periods at each of two temperatures, a low one (12° ± 1° C.), favorable for the growth of wheat, and one too high for the best development of even the spring variety (21° ± 1°). Temperature control was maintained from the first of December until early April.

The long day hastened the development of both varieties, but the acceleration was much more pronounced in the case of the spring variety. The short day retarded the development of both varieties.

At the low temperature, the short day increased leaf size, head length, and generally the number of tillers, and decreased kernel weight and yield of the plants of both varieties, causing complete sterility in Turkey in two out of three experiments. The long day decreased dry weight, tillering, head length, and total yield of grain in both varieties, although Turkey's yield per head and average kernel weight were always highest at this day length. Yield per plant of this variety at the long day was reduced because of the reduction in tillering.

The short day was more damaging than the long day to the yield of grain in Turkey, whereas the long day was the more damaging to Hard Federation.

The high temperature was more damaging to the winter variety than it was to the spring variety, heading of the former being sparse and the growth of the plants abnormal above 20° C.

LITERATURE CITED

(1) Adams, J.
1923. The effect on certain plants of altering the daily period of light. Ann. Bot. [London] 37: 75–94.

(2) ———
1924. Does light determine the date of heading out in winter wheat and winter rye? Amer. Jour. Bot. 11: 535–539.

(3) ———
1924. Duration of light and growth. Ann. Bot. [London] 38: [509]–523.

(4) ———
1925. Some further experiments on the relation of light to growth. Amer. Jour. Bot. 12: 398–412.

(5) Arthur, J. M., Guthrie, J. D., and Newell, J. M.
1930. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. 17: 416–482, illus.

(6) Bayles, B. B., and Martin, J. F.
1931. Growth habit and yield in wheat as influenced by time of seeding. Jour. Agr. Research 42: 483–500, illus.

(7) Berkley, E. E.
1931. Studies of the effects of different lengths of day, with variations in temperature, on vegetative growth and reproduction in cotton. Ann. Missouri Bot. Gard. 18: 573–602, illus.

(8) Chmelar, F., and Mostovoj, K. I.
1929. A contribution towards the distinguishing of winter, spring and alternative habits of growth of cereals (specially wheat) in [the] laboratory. Věstnik Ceskosl. Akad. Zeměd. 5: 10–16, illus. [German and English summaries, p. 13–16.]

(9) Cooper, H. P.
1923. The inheritance of the spring and winter growing habit in crosses between typical spring and typical winter wheats, and the response of wheat plants to artificial light. Jour. Amer. Soc. Agron. 15: 15–25.

(10) Doroshenko, A. V.
1927. Photoperiodism of some cultivated forms in connection with their origin. Trudy Prikl. Bot. i Selek. (Bul. Appl. Bot. and Plant Breeding) 17 (1): [167]–220, illus. [In Russian. English summary, p. 218–219.]

(11) ——— and Rasumov, V. I.
1929. Photoperiodism of some cultivated forms in connection with their geographical origin. Trudy Prikl. Bot. i Selek. (Bul. Appl. Bot. and Plant Breeding) 22 (1): 219–273. [In Russian. English summary, p. 274–276.]

(12) Enomoto, N.
1929. On the physiological difference between the spring and winter types in wheat and barley. Jour. Imp. Agr. Expt. Sta. Tokyo 1: 107–138, illus. [In Japanese. English summary, p. 136–138.]

(13) Forster, H. C., Tincker, M. A. H., Vasey, A. J., and Wadham, S. M.
1932. Experiments in England, Wales and Australia on the effect of length of day on various cultivated varieties of wheat. Ann. Appl. Biol. 19: 378–412, illus.

(14) Garner, W. W., and Allard, H. A.
1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Research 18: 553–606, illus.

(15) ——— and Allard, H. A.
1923. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Research 23: 871–920, illus.

(16) Gassner, G.
1918. Beiträge zur physiologischen charakteristik sommer- und winterannueller gewächse, insbesondere der getreidepflanzen. Ztschr. Bot. 10: [417]–480, illus.

(17) GILBERT, B. E.
1926. INTERRELATION OF RELATIVE DAY LENGTH AND TEMPERATURE. Bot. Gaz. 81: 1–24, illus.

(18) ———
1926. THE RESPONSE OF CERTAIN PHOTOPERIODIC PLANTS TO DIFFERING TEMPERATURE AND HUMIDITY CONDITIONS. Ann. Bot. [London] 40: 315–320.

(19) HURD-KARRER, A. M.
1930. THE FORMATIVE EFFECT OF DAY LENGTH ON WHEAT SEEDLINGS. Jour. Md. Acad. Sci. 1: 115–126, illus.

(20) HUTCHESON, T. B., and QUANTZ, K. E.
1917. THE EFFECT OF GREENHOUSE TEMPERATURES ON THE GROWTH OF SMALL GRAINS. Jour. Amer. Soc. Agron. 9: 17–21, illus.

(21) KELLERMAN, K. F.
1926. A REVIEW OF THE DISCOVERY OF PHOTOPERIODISM: THE INFLUENCE OF THE LENGTH OF DAILY LIGHT PERIODS UPON THE GROWTH OF PLANTS. Quart. Rev. Biol. 1: 87–94, illus.

(22) KOLTZOV, L. I.
1923. [INFLUENCE OF THE DURATION OF ILLUMINATION ON GROWTH AND DEVELOPMENT OF PLANTS.] Ann. State Inst. Expt. Agron. 1: 139–142. [In Russian. English translation in Bureau Plant Industry Library, Washington, D. C.]

(23) LUBIMENKO, V. N., and SZÈGLOVA, O. A.
1928. L'ADAPTATION PHOTOPÉRIODIQUE DES PLANTES. Rev. Gén. Bot. 40: [513]–536, [577]–590, [675]–689, 747–768, illus.

(24) MCKINNEY, H. H., and SANDO, W. J.
1930. THE BEHAVIOR OF WINTER WHEAT IN ARTIFICIAL ENVIRONMENTS. Science (n. s.) 71: 668–670.

(25) MAXIMOV, N. A.
1925. [IMPORTANCE IN THE LIFE OF THE PLANT OF THE RATIO BETWEEN LENGTH OF DAY AND LENGTH OF NIGHT (PHOTOPERIODISM).] Trudy Prikl. Bot. i Selek. (Bul. Appl. Bot. and Plant Breeding) 14(5): 69–90. [In Russian. English translation in Bureau Plant Industry Library, Washington, D. C.]

(26) ———
1929. EXPERIMENTELLE ÄNDERUNGEN DER LÄNGE DER VEGETATIONSPERIODE BEI DEN PFLANZEN. Biol. Zentbl. 49: 513–543, illus. [Also in Trudy Prikl. Bot. i Selek. (Bul. Appl. Bot. and Plant Breeding) 20: 169–209.]

(27) ———
1930. PHYSIOLOGICAL CONTROL OF THE LENGTH OF THE VEGETATIVE PERIOD. 5th Internatl. Bot. Cong. Cambridge, Abs. Communications, p. 275–276.

(28) ———
1930. A TEXTBOOK OF PLANT PHYSIOLOGY. Translated from the Russian; edited by A. E. Murneek and R. B. Harvey. 381 p., illus. New York.

(29) ——— and POJARKOVA, A. I.
1925. ÜBER DIE PHYSIOLOGISCHE NATUR DER UNTERSCHIEDE ZWISCHEN SOMMER UND WINTERGETREIDE. Jahrb. Wiss. Bot. 64: 702–730, illus. [Also in Trudy Prikl. Bot. i Selek. (Bul. Appl. Bot. and Plant Breeding) 14(1): 211–234.

(30) MURINOV, A. D.
1913. [DAS AEHRENTREIBEN DES WINTERROGENS UND WINTERWEIZENS BEI FRÜHJAHRSAUSSAAT.] Zhur. Opytn. Agron. IUgo-Vostoka (Jour. Expt. Landw. Südost. Eur.-Russlands 14: 238–254. [In Russian. German summary, p. 254.]

(31) PERCIVAL, J.
1921. THE WHEAT PLANT; A MONOGRAPH. 463 p., illus. London.

(32) POJARKOVA, A. J.
1927. TEMPERATURBEDINGUNGEN DER KEIMUNG ALS BESTIMMENDE FAKTOR FÜR ÄHRENBILDUNG BEIM WINTERGETREIDE. Ber. Deut. Bot. Gesell. 45: 627–637.

(33) RASUMOV, V. J.
1930. ÜBER DIE PHOTOPERIODISCHE NACHWIRKUNG IM ZUSAMMENHANG MIT DER WIRKUNG VERSCHIEDENER AUSSAATTERMINE AUF DIE PFLANZEN. Planta, Arch. Wiss. Bot. 10: [345]–373, illus.

(34) REDINGTON, G.
1929. THE EFFECT OF THE DURATION OF LIGHT UPON THE GROWTH AND DEVELOPMENT OF THE PLANT. Cambridge Phil. Soc. Biol. Rev. and Biol. Proc. 4: 180–208.
(35) RUSSELL, E. J.
1932. SOIL CONDITIONS AND PLANT GROWTH. Ed. 6, 636 p., illus. London, New York [etc.].
(36) TINCKER, M. A. H.
1925. THE EFFECT OF LENGTH OF DAY UPON THE GROWTH AND REPRODUCTION OF SOME ECONOMIC PLANTS. Ann. Bot. [London] 39: [721]–754, illus.
(37) ———
1929. THE VARIETAL RESPONSE OF THE PLANT TO THE LENGTH OF DAY. Conf. Emp. Meteor. (Agr. Sect.) Papers and Discussions 1929: 34–39.
(38) WACAR, B. A.
1925. ZUR FRAGE ÜBER D. EINFLUSS TEMPERATUR AUF DIE BILDUNG DER AEHREN BEI WINTERROGEN UND WINTERWEIZEN. Jour. Landw. Wiss. Moskau 2: [776]–785, illus. [In Russian. German summary, p. 785.]
(39) WANSER, H. M.
1922. PHOTOPERIODISM OF WHEAT; A DETERMINING FACTOR IN ACCLIMATIZATION. Science (n. s.) 56: 313–315.
(40) WEAVER, J. E., and HIMMEL, W. J.
1929. RELATION BETWEEN THE DEVELOPMENT OF ROOT SYSTEMS AND SHOOT UNDER LONG AND SHORT DAY ILLUMINATION. Plant Physiol. 4: 435–457, illus.

EFFECTS OF CHLORINE, BROMINE, AND FLUORINE ON THE TOBACCO PLANT[1]

By LLOYD B. WILSON[2]

Formerly *Agent, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

A considerable body of data is available on the influence of the element chlorine, applied as chlorides, upon the growth of crop plants. No special investigation, however, appears to have been made of the influence of the closely related elements, bromine and fluorine. The object of much of the work with chlorine has been to determine whether chlorides are beneficial or harmful to the crop. The literature on this subject, as reviewed by Tottingham (*10*) [3] and by Lomanitz (*6*), shows that it has long been the practice among growers to apply sodium chloride as a fertilizer material and that the effect of chlorides depends partly on such factors as the quantity of chlorine applied; the kind of crop; the type, reaction, and moisture content of the soil; and the meteorological conditions prevailing during the growing season.

The influence of chlorine on tobacco has received careful study by Garner and his associates (*4*). In comparative tests with sulphate of potash and chloride of potash on Durham sandy loam, these investigators found an average increase of 10 per cent in yield on plots to which chlorides were applied. Two reasons are given for the stimulatory effect of chlorides: (1) Chlorine increases the solubility of magnesium, enabling the plant to absorb enough of this element to meet its nutritional requirements; and (2) the presence of chlorides within the tobacco plant enables it, by maintaining an increased water content, to better withstand periods of drought. Garner and his associates noted, moreover, that plants supplied with an excess of chlorine were dwarfed and that their leaves were glossy, extremely thick, and brittle. In extreme cases the margins of the leaves were curled upward; such leaves were found to have an abnormally high water content and to be gorged with starch. These workers also found that an excess of chlorine lowers the market value of the cured leaf through its deleterious effects on color, elasticity, and keeping quality. The manufactured product may be affected also, since tobacco of excessive chlorine content is high in moisture and low in organic acids and hence does not burn readily.

Since, so far as is known, no one has investigated the possible beneficial or harmful effects of the elements bromine and fluorine upon the tobacco plant and since no detailed study seems to have been made of the effects of chlorine, bromine, or fluorine upon the anatomy of the leaves or upon the physicochemical properties of the sap, an investigation of these matters was undertaken and is reported herein.

---

[1] Received for publication Dec. 28, 1932; issued June, 1933. Cooperative investigation by the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Carolina Department of Agriculture.

[2] The writer wishes to acknowledge the cooperation of Frederick A. Wolf, Duke University, Durham, N. C., in this investigation.

[3] Reference is made by number (italic) to Literature Cited, p. 899.

MATERIAL AND METHODS

Material was secured both from tobacco plants (*Nicotiana tabacum* L.) grown in the field and from plants grown under glass. The field-grown material was collected in two localities, one about 14 miles north of Durham, N.C., and the other about 16 miles south of Durham. The plants in the greenhouse were grown in 6-inch porous pots in a soil consisting of a mixture of loam and river-washed sand. A small quantity of 8-4-4 fertilizer was stirred into the soil before the plants were transplanted. The soil was kept moist by applications of dilute solutions of salts interspersed with applications of tap water. Each lot of plants, except the controls, was given applications of one of the following salts: Amonium chloride (NH_4Cl), magnesium chloride ($MgCl_2$), potassium chloride (KCl), sodium chloride (NaCl), ammonium bromide (NH_4Br), sodium bromide (NaBr), potassium bromide (KBr), ammonium fluoride (NH_4F), potassium fluoride (KF), or sodium fluoride (NaF). Saucers placed beneath the pots prevented loss of salts by drainage and permitted the salts to accumulate in increasing concentrations. When incipient collapse indicated that the plants could tolerate no more salts, suitable moisture content was maintained by tap water alone. A canopy of cheesecloth was used to provide partial shade.

Material for anatomical study was cut from the leaves by means of a cork borer and was fixed in formalin-acetic-alcohol solution. Paraffin sections were cut 20μ to 30μ in thickness and were stained with Heidenhain's iron-alum haematoxylin.

Material for physicochemical studies was collected from the field between 8 and 9 a. m., and from the greenhouse at about 7.30 a. m. Each sample from the field consisted of representative leaves taken from a dozen or more plants. The samples were immediately packed in solid carbon dioxide in cardboard cartons, which were then sealed with tape. The material from the greenhouse was placed in thermos jugs and taken directly to a cold room maintained at 38° F. The sap was extracted from the samples by grinding the leaves in a meat chopper. Suspended matter was removed from the expressed sap by centrifugalization, and the moisture content was determined by means of a refractometer.

Such physicochemical properties as depression of the freezing point, osmotic concentration, and percentage of bound water were determined by the standard method (*8*). In this method, Δ equals the freezing-point depression of the sap as determined by the Beckmann thermometer and Δa equals the freezing-point depression of a volume of sap sufficient to contain 20 g of water, in which 6.845 g of sucrose (a molar concentration) has been dissolved. The difference between these two values is expressed by the formula

$$\Delta a - \Delta = \Delta s.$$

If, then, the depression of the freezing point of a molar solution of sucrose alone is subtracted from Δs, this value equals Δx. From these values the bound water content, or hydrophilic colloidal content, of the sap is calculated by means of the following formula:

$$\Delta x.89.2 \left(\frac{\text{Percentage of free water in a molar solution of sucrose}}{\Delta s} \right) = \text{Percentage of bound water.}$$

EFFECTS OF HALOGEN SALTS ON THE TOBACCO PLANT

Tobacco responds readily to applications of salts containing chlorine, bromine, or fluorine, but the effects of the various compounds differ somewhat. When a mixed fertilizer containing 4 or 5 per cent of

FIGURE 1.—Tobacco leaves showing injury from excessive application of chlorides

potash as potassium chloride was applied to tobacco plants in the field, these plants became stunted and their leaves were characteristically thickened and curled along the margin (fig. 1), thus confirming

the observations of Garner and his associates (*4*). In the greenhouse, in a soil which was deficient in nitrogen, potash, and phosphoric acid but to which chlorides were applied in excess, tobacco plants

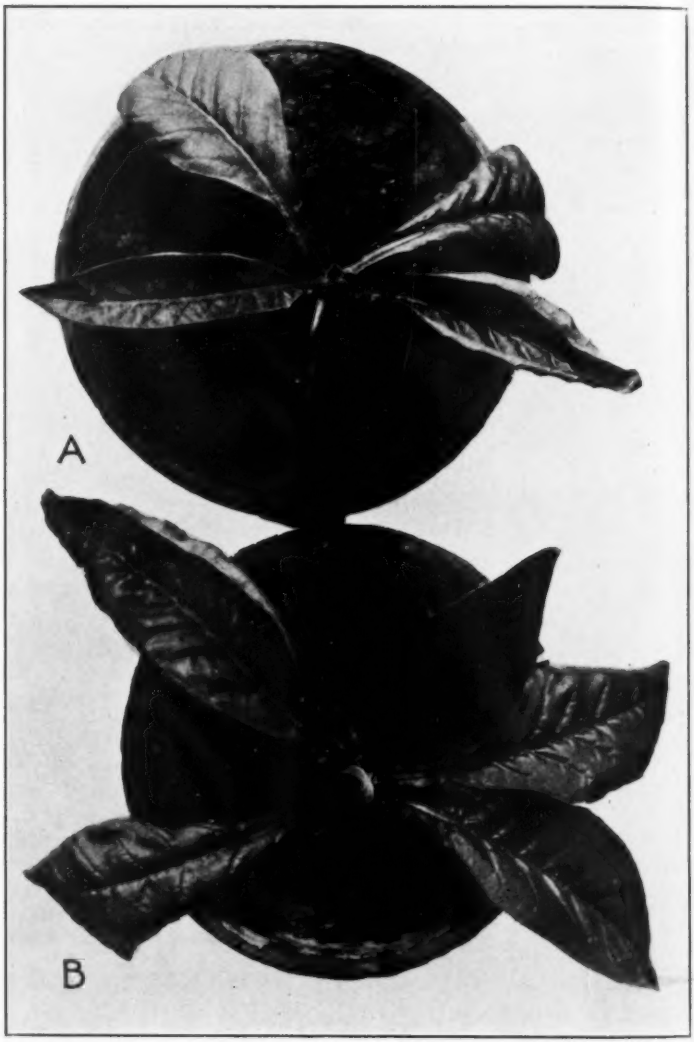

FIGURE 2.—Tobacco grown in the presence of bromides: A, Plant given applications of ammonium bromide; B, plant given applications of potassium bromide

grew less rapidly than did the controls. Instead of being thin and pliable, as normally, the leaves were thick and stiff. There was no curling upward of the leaf margins after applications of the chlorides of magnesium, potassium, or sodium; curling occurred, however, after

applications of ammonium chloride. From this effect it would appear that the cumulative action of an excess of chlorine alone does not cause curling. The fact that plants receiving applications of ammonium chloride made a more vigorous growth and had larger leaves than did those receiving the other chlorides indicates that the marginal curling of leaves may be conditioned by the availability of nitrogen and is therefore correlated with rate of growth.

Plants receiving applications of bromine as bromides of ammonium, sodium, or potassium developed leaves that were thick, shiny, and

FIGURE 3.—Tobacco plant to which ammonium fluoride was applied

brittle. Curling of the leaf margin resulted from the use of ammonium bromide or potassium bromide (fig. 2), but not from the use of sodium bromide.

When permitted to accumulate in excessive amounts, fluorine produces a more pronounced inhibition of growth than does either chlorine or bromine; and it seems, in addition, to have a toxic effect. Marked thickening occurred after applications of fluorides of ammonium, calcium, potassium, or sodium; but there was no rolling upward of the leaf margins except after applications of ammonium fluoride. (Fig. 3.)

These results indicate that excessive amounts of chlorine, bromine, or fluorine interfere with the normal growth and expansion of tobacco leaves and with the elongation of the stems. From the chemical similarity of these elements, it may be assumed that their salts would have similar effects on cellular activities. Evidence of similarity in physiological effects on tobacco is shown by anatomical studies.

EFFECT ON ANATOMY OF LEAVES

Normal tobacco leaves embedded in paraffin were cut transversely in sections of sufficient thickness to include entire cells. Leaves from

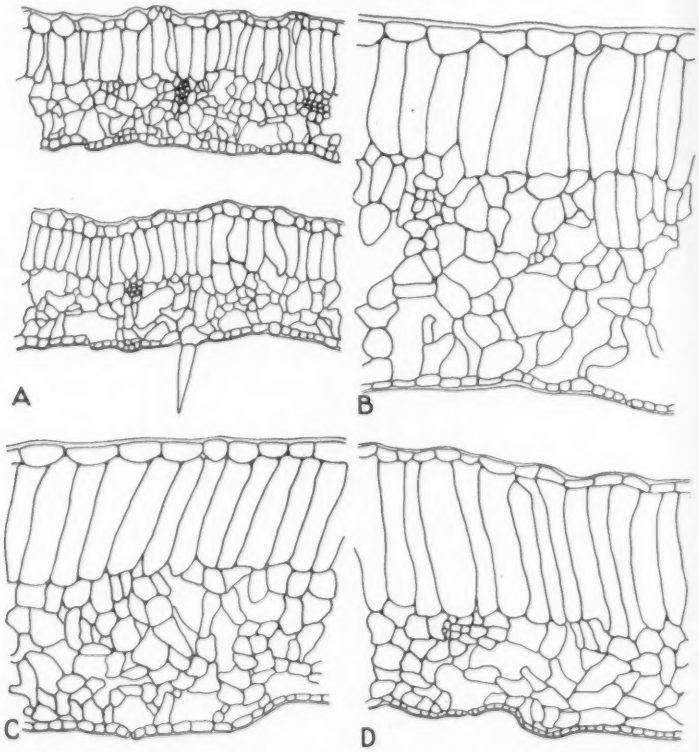

FIGURE 4.—Cross section of tobacco leaves showing thickening produced by halogen salts: A, Normal leaf; B, leaf injured by chlorine; C, leaf injured by bromine; D, leaf injured by fluorine

plants that had been given excessive applications of halogen salts were similarly embedded and sectioned. Microscopic examination of these sections showed that the salts had caused an increase in the thickness of the leaves and that this increase was due not to an increase in the number of cells but to the enlargement of cells of all tissues except, perhaps, those of the vascular bundles.

Figure 4 shows cross sections of leaves drawn to the same scale with the aid of a microprojector and indicates that leaves from plants

to which chlorides had been applied were approximately two and one-half times as thick as normal leaves, and that those to which bromine and fluorine had been applied were approximately twice as thick as normal leaves.

Garner and his associates (*4*) have shown that the increased thickness and brittleness of leaves of plants receiving an excess of chlorides is primarily due not to toxicity but to an interference with the carbohydrate metabolism in which the water relations are involved. As already stated, these investigators noted that an excess of chlorine causes the leaves to become gorged with starch. In earlier studies (*2*) on the curing of tobacco they reported that the partial wilting of the green leaf has a decided effect in stimulating amylolytic processes involved in the conversion of starch to reducing sugars. In the same year (1914), a similar starch-water relationship in plant tissues was noted by Lundegårdh (*7*), who found, furthermore, that an increase in water content causes starch to re-form. More recently Schroeder and Horn (*9*) and Bruns (*1*), in investigations on several species of plants, have confirmed the observation that starch is digested in cells which are not turgid and that it is re-formed when the cells have again absorbed sufficient water to become turgid.

The presence of chlorides in excessive amounts should therefore tend to maintain a high state of turgidity within the cells of tobacco leaves. It seems probable that bromides and fluorides would have a similar effect.

EFFECT ON PHYSICOCHEMICAL PROPERTIES OF SAP

In order to ascertain whether halogens modify the osmotic concentration of the sap and whether these changes are correlated with increased size of the leaf cells, determinations were made of the freezing-point depression, osmotic concentration, and bound-water percentage of extracted sap. The results of a series of such determinations are given in Table 1.

TABLE 1.—*Effects of various halogen salts on physicochemical properties of the sap of leaves of tobacco plants grown in the greenhouse in 1931*

Date of analysis	Solution applied	Freezing-point depression		Osmotic concentration	Bound water [c]
		Δ[a]	Δ[a][b]		
		° *C.*	° *C.*	*Atmospheres*	*Per cent*
July 9	Control (tap water)	0.682	2.209	8.22	0
	KBr	.875	2.360	10.54	0
	NH_4Br	.814	2.291	9.80	0
	KCl	1.092	2.845	13.14	0
	NaCl	.981	2.469	11.89	0
	NH_4Cl	.941	3.046	11.33	0
July 21 and 22	Control (tap water)	.721	2.873	8.68	1.28
	KBr	1.085	3.201	13.06	0
	NaBr	1.027	2.784	12.36	0
	NH_4Br	.915	3.041	11.02	.21
	KCl	1.106	3.312	13.31	3.44
	NaCl	1.021	3.222	12.29	3.24
	NH_4Cl	.903	3.064	10.87	1.65
	NaF	.788	2.992	9.49	3.36

[a] Freezing-point depression as determined by the Beckmann thermometer.
[b] Freezing-point depression of a volume of sap sufficient to contain 20 g of water in which 6.845 g of sucrose has been dissolved.
[c] Computed from freezing-point-depression values. (See p. 890.)

TABLE 1.—*Effects of various halogen salts on physicochemical properties of the sap of leaves of tobacco plants grown in the greenhouse in 1931*—Continued

Date of analysis	Solution applied	Freezing-point depression		Osmotic concentration	Bound water
		Δ	Δ*a*		
		° *C.*	° *C.*	*Atmospheres*	*Percent*
Aug. 3	Control (tap water)	0.708	2.895	8.53	2.81
	KBr	1.082	3.250	13.02	2.06
	NaCl	1.009	3.181	12.15	2.22
	NaF	.874	3.056	10.52	2.62
Aug. 14	Control (tap water)	.817	3.043	9.84	4.25
	NH_4Br	.876	3.079	10.55	3.36
	KCl	1.126	3.288	13.55	1.73
	NaF	.959	3.192	11.55	4.51
Average	Control (tap water)	.732	2.755	8.82	2.05
	KBr	1.014	2.937	12.21	.69
	NaBr	1.027	2.784	12.36	0
	NH_4Br	.868	2.804	10.46	1.19
	KCl	1.108	3.148	13.33	1.72
	NaCl	1.004	2.957	12.11	1.82
	NH_4Cl	.922	3.055	11.10	.83
	NaF	.874	3.080	10.52	3.50

These data show that sap from the leaves of plants receiving excessive amounts of salts had larger values for Δ and Δ*a* than that from the leaves of plants receiving tap water only, or that the cell sap of plants to which salts had been applied had the greater osmotic concentration.

Garner and his associates (*3*) have shown that there is a progressive increase in hydrogen-ion concentration in the sap of tobacco leaves as the leaves approach maturity. Since active acidity is correlated with changes in osmotic concentration there should also be a gradual increase in osmotic concentration in plants that are approaching maturity and that have received applications of salts. The data in Table 1 indicate a tendency toward increased osmotic concentration with increasing age.

The ability of the leaf to bind water is influenced by an excess of salts, and also by the age of the leaf as indicated by the absence of bound water in all samples on July 9 (Table 1) and by its occurrence in nearly all later samples. Further evidence of the influence of age as a factor in increasing the binding of water was strikingly exhibited by the difficulty with which sap could be extracted from leaves gathered from the field early in October, 1931. The present data, with the exception of the results obtained when the plants were given sodium fluoride, show that the bound-water content of normal leaves is higher than that of the leaves of plants to which chlorides or bromides has been applied. The physicochemical effects produced by sodium fluoride indicate that the toxicity of this salt may possibly have interfered with the absorption of water, thus causing more than the normal quantity of free water to be transformed to bound water and producing a condition similar to that which occurs during drought. While studying drought resistance in cereals, Harris and his coworkers, in an investigation cited by Gortner (*5, p. 235–236*), noted that as long as there is an abundance of moisture there is little tendency for the development of hydrophilic colloids and the elaboration

of bound water but that with the onset of conditions of stress, as of drought, water is transformed from the free to the bound state.

The moisture supply of tobacco plants growing in the field fluctuates over a wide range during the season. In the present experiments the moisture supply of plants grown in the greenhouse was maintained more nearly at the optimum. In physicochemical properties the sap of plants grown in the field may therefore differ from that of those grown under glass. Data bearing on this question were secured by comparing the results of the greenhouse experiments (Table 1) with results obtained from tobacco leaves collected in the field in each of the two localities previously mentioned, namely, area 1, north of Durham, N. C., and area 2, south of Durham (Table 2).

TABLE 2.—*Effects of chlorides on physicochemical properties of the sap of leaves of tobacco plants grown near Durham, N. C., 1931*

Date of analysis	Effect of indicated quantity of chlorides on—							
	Freezing-point depression				Osmotic concentration		Bound water [c]	
	Δ [a]		Δa [b]					
	Normal Cl	Excess Cl	Normal Cl	Excess Cl	Normal Cl	Excess Cl	Normal Cl	Excess Cl
	° *C.*	° *C.*	° *C.*	° *C.*	*Atmospheres*	*Atmospheres*	*Per cent*	*Per cent*
June 11	0.968	0.982	3.237	3.262	11.66	11.82	3.89	4.30
June 23	.590	.591	2.800	2.765	7.11	7.12	2.74	1.31
July 10	.706	.676	2.250	2.175	8.50	8.14	0	0
July 24	.828	.803	3.214	3.041	9.97	9.67	11.18	6.02
Average	.773	.763	2.875	2.811	9.31	9.19	4.45	2.90
AREA 2 (SOUTH OF DURHAM)								
June 18	0.618	0.567	2.842	2.718	7.45	6.83	3.29	0.37
June 24	.785	.848	3.096	3.087	9.45	10.21	6.52	3.86
July 11	.643	.643	2.130	2.024	7.75	7.75	0	0
July 25	.891	.666	3.160	2.847	10.73	8.02	7.15	3.84
Average	.734	.681	2.807	2.669	8.85	8.20	4.24	2.02

[a] Freezing-point depression as determined by the Beckmann thermometer.
[b] Freezing-point depression of a volume of sap sufficient to contain 20 g of water in which 6.845 g of sucrose has been dissolved.
[c] Computed from freezing-point-depression values. (See p. 890.)

In each of these two areas tobacco leaves were collected from two fields, in one of which chlorides had been applied in excess, whereas in the other only the normal 2 per cent or less of chlorides in the fertilizer had been applied. The chlorine-injured leaves selected for examination, all of which were taken from stunted plants, were curled and exceedingly brittle.

Table 2 shows that on corresponding dates the freezing-point depression, the osmotic concentration, and the bound-water percentage were, in general, greater in the normal leaves than in those injured by an excess of chlorides.

Moreover, the osmotic-concentration values of field-grown plants to which excessive applications of chlorides were made (Table 2) were not the same as those of plants grown under glass in the presence of an excess of sodium chloride or potassium chloride (Table 1). Such differences may be explained by the fact that there was less fluctuation in the water supply of plants grown under glass than in that of plants grown in the field. Since "normal" plants (Table 2) were liberally supplied with essential minerals and the controls (Table 1) were grown in a soil deficient in minerals, the properties of the sap of these normal and control plants can not be expected to accord.

It might be expected that the values for normal leaves in Table 2 would exceed those of leaves injured by chlorine. The apparent inconsistencies may be partly due to lack of correspondence in age of the normal and the chlorine-injured plants. Both normal and chlorine-injured plants from which leaves were taken for the analyses recorded in Table 2 were transplanted at approximately the same date. In both tables is shown a tendency toward increase in osmotic concentration with increase in age of plants. It is well known among growers that the leaves of plants given an excess of chlorine mature later, by two weeks or more, than those of normal plants. Despite the apparent equality in age, the chlorine-injured plants remained considerably smaller and hence were physiologically younger than normal plants set at the same date.

Moreover, as previously stated (*4*), it has been found that excess of chlorine results in a low organic-acid content. The differences between the osmotic-concentration values of the sap of normal leaves and those of the sap of leaves of chlorine-injured plants may, therefore, be occasioned by the relatively high acid content of normal leaves and the low acid content of the chlorine-injured ones.

So far as bound water is concerned, all data are in agreement in showing that there is relatively little tendency for the transforming of water from a free to a bound condition when chlorine is present in excessive amounts.

SUMMARY

Previous investigations have shown that moderate applications of chlorine stimulate the growth and increase the yield of tobacco. An excess of chlorine, however, interferes with normal carbohydrate metabolism and causes the leaves to become thick and brittle and to curl upward at the margins. Such leaves have a high starch content, a high water content, and a low organic-acid content.

The present anatomical studies of leaves from normal tobacco plants as compared with leaves from plants to which certain chlorides, bromides, or fluorides were applied show that these elements cause the leaves to become thickened by enlargement of the leaf cells. When the concentration of the halides is maintained at approximately the limit of tolerance, chlorine causes greater enlargement than does bromine or fluorine.

Experiments with plants grown under glass in the presence of an excess of a single salt show that chlorine, bromine, or fluorine increases the osmotic concentration of expressed sap, as measured by depression of the freezing point. Increased osmotic concentration in tobacco is therefore correlated with enlargement of the leaf cells. The osmotic-concentration values tend to increase with the age of the plant.

The leaves of tobacco plants to which excessive amounts of chlorides or bromides have been applied have less bound water than the leaves of plants grown in a medium deficient in mineral nutrients.

Tobacco responds to sodium fluoride by transforming free water to bound water in such quantities as to indicate that this compound induces a state of physiological drought.

The sap of tobacco plants grown in the field in the presence of excessive amounts of chlorides had a lower osmotic concentration and less bound water than the sap of field-grown plants to which a liberal application of a complete fertilizer had been given. The lower values of the sap of chlorine-injured leaves may be partly explained by physiological immaturity and low organic-acid content.

LITERATURE CITED

(1) Bruns, A.
1925. untersuchungen zur auffindung der ursache der amylum verminderung-beschleunigung in welkenden laubblatt. Bot. Arch. 11 : 40–103.

(2) Garner, W. W., Bacon, C. W., and Foubert, C. L.
1914. research studies on the curing of leaf tobacco. U. S. Dept. Agr. Bul. 79, 40 p.

(3) ——— McMurtrey, J. E., Jr., Bacon, C. W., and Moss, E. G.
1923. sand drown, a chlorosis of tobacco due to magnesium deficiency, and the relation of sulphates and chlorids of potassium to the disease. Jour. Agr. Research 23 : 27–40, illus.

(4) ——— McMurtrey, J. E., Jr., Bowling, J. D., and Moss, E. G.
1930. rôle of chlorine in nutrition and growth of the tobacco plant and its effect on the quality of the cured leaf. Jour. Agr. Research 40 : 627–648, illus.

(5) Gortner, R. A.
1929. outlines of biochemistry, the organic chemistry and the physico-chemical reactions of biologically important compounds and systems. 793 p., illus. New York and London.

(6) Lomanitz, F.
1924. the influence of sodium chloride upon alfalfa grown in solution cultures. Soil Sci. 18 : 353–368, illus.

(7) Lundegårdh, H.
1914. einige bedingungen der bildung und auflösung der stärke. ein beitrag zur theorie des kohlenhydratstoffwechsels. Jahrb. Wiss. Bot. 53 : [421]–463.

(8) Newton, R., and Martin, W. M.
1930. physico-chemical studies of the nature of drought resistance in crop plants. Canad. Jour. Research 3 : 336–383, 385–427, illus.

(9) Schroeder, H., and Horn, T.
1922. das gegenseitige mengenverhältnis der kohlenhydrate in laubblatt in seiner abhangigkeit vom wassergehalt. Biochem. Ztschr. 130 : [165]–198.

(10) Tottingham, W. E.
1919. a preliminary study of the influence of chlorides on the growth of certain agricultural plants. Jour. Amer. Soc. Agron. 11 : 1–32.

179377—33——3

INFECTIOUS VARIEGATION IN THE APPLE [1]

By F. C. BRADFORD, *Research Associate in Horticulture, Michigan Agricultural Experiment Station*, and LLOYD JOLEY, *Student, Michigan State College* [2]

INTRODUCTION

The variegation of apple leaves discussed in this paper appeared as an unexpected complication in an experiment on double working, raising new questions which had to be answered before the study as originally planned could proceed with any prospect of definite results. Since this secondary study has reached a point where separation of horticultural and pathological aspects seems possible, and since it deals with a matter which has received rather scant attention, its salient points are placed on record, together with such historical data as are available.

HISTORY OF THE DISEASE

Under various names, variegation of apple leaves has been mentioned several times in the literature. Stewart (*12*),[3] in 1910, described sporadic cases found in New York State since 1896. In 1915 Clinton (*5*) described a manifestation in Connecticut which may have been identical. In the following year Morse (*9*) reported, as a leaf trouble "new to Maine" the occurrence of chlorotic areas in leaves of Baldwin, Northern Spy, and Harvey. The appearance of the leaves shown in his photographs is in every way similar to that of the diseased leaves discussed in this paper. Blodgett and others [4] have reported as "apple mosaic" additional cases in New York, and have demonstrated that the disease is transmissible by budding and grafting. Blodgett states [5] that specimen leaves from material used in the study here reported "certainly look identical" with the trouble he reported, that Whetzel has collected similar specimens in Michigan, and that others who have seen his specimens have recalled seeing similar cases in other States.

In northern Germany Braun (*2*) reports as "Buntblätterigkeit" or "Panaschierung" the occurrence in 1928 of "white spots in otherwise sound green leaves" in scattered trees of the apple varieties Fettapfel and Rosenapfel.

Though a rather extensive search of the classic papers on infectious variegation yields no mention of this disease in the apple, it is not to be inferred that it is particularly new. Inasmuch as the early European reports have been overlooked or discredited, and inasmuch as they should constitute a rather important chapter in the history of infectious variegation, a detailed statement is presented here.

---

[1] Received for publication Sept. 8, 1932; issued June, 1933. Journal article No. 116 (U. S.) from the Michigan Agricultural Experiment Station.

[2] The writers acknowledge their indebtedness to W. C. Dutton, of the Michigan Agricultural Experiment Station, for the photograph used and to Dr. Ray Nelson, of the Michigan station, and Dr. F. M. Blodgett, of Cornell University, for references to American phytopathological literature.

[3] Reference is made by number (italic) to Literature Cited, p. 908.

[4] ORTON, C. R., and WOOD, J. I. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1923. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 33 : 82. 1924.

[5] BLODGETT, F. M. Correspondence. Aug. 15, 1932.

In 1867 Bossin (*1*), without giving citation, stated that Vibert had described a variety of apple with variegated leaves. This, according to Bossin's recollection, occurred about 1827. Bossin made no mention of the variegation being infectious, though the nature of his topic gave him occasion to note a fact of this nature.

In 1830 Sageret (*11*) stated that he had read in the Philosophical Transactions an account (presumably Cane's (*3*) published in 1720, describing an observation made in 1692) of transmission of variegation from scion to stock in jasmine. In 1825 Noisette (*10*), whose nursery was near Paris, had reported observing, on several occasions, transmission of variegation from scion to stock in several kinds of woody plants, which he did not specify. Apparently Sageret had not seen Noisette's statement, for he mentioned the account of the jasmine without, as he said later (reported by Duchartre (*6*)), giving it full credence, because he knew of no parallel case. Vibert, upon seeing Sageret's statement concerning the jasmine, reported to him that he had seen transmission of variegation in the apple. Some years later Vibert (*13*) gave an account of this occurrence, as follows:

> Some time ago, and during several years, I have seen, at the establishment of a brother of Noisette, the nurseryman, at La-Queue-En-Brie, apple trees which, having been shield-budded with varieties with variegated leaves, and their grafts failing from any cause whatever, none the less produced branches with variegated leaves. Astonished at this strange anomaly, I repeated at home the same operation, on Paradise, using as scion the pommier à feuilles d'Aucuba. In the following spring I completely destroyed all my scions, and all the stocks produced shoots with leaves more or less variegated, even those whose grafts had perished after being inserted.

Vibert recorded at the same time a similar subsequent experience with the rose on dog-rose stock.

In 1839, according to Leroy (*7, v. 3, p. 301–302*), the nurseryman Noisette gave the initial description of the "pommier a feuilles d'Aucuba," so called because of its variegated leaves, and Leroy secured specimen trees from the vicinity of Paris at that time. Noisette's mention in 1825 of transmission, his initial description of a variegated variety, and his brother's experience seem to warrant the belief that the apple was one of the plants on which he based his 1825 report. If it was not, Vibert had demonstrated transmission in the apple by 1835, when Sageret reported Vibert's statement, as mentioned by Duchartre (*6*).

Sageret, having been somewhat sceptical of the jasmine case, encountered similar scepticism of his own report concerning the apple. Morren (*8*) stated some years later:

> Pyrame de Candolle, Poiteau, and others of the time attached true importance to it, but without being convinced of its authenticity. Sageret's observation was never cited except with hesitation. The new facts [i.e., those available in 1870] established the truth of the phenomenon in an incontestable manner.

Apparently Sageret's report came too early. Had it been made in 1870 its historical importance in antedateing nearly all of the better known cases would undoubtedly have gained wider recognition.

Other reports indicating infectious variegation in the apple are rare and vague. A specimen submitted by Simirenko, as reported by Carrière and André (*4*) in 1889 suggests a manifestation of infectious variegation, but is not evidence. Consideration should, however, be given the fact that trees raised in well-conducted nurseries have little opportunity of manifesting variegation in the seedling roots where the

potentiality for manifestation is greater. Leroy cultivated the pommier à feuilles d'Aucuba in his nursery at Angers, and stated in 1873 that it was more notable for its variegation than valuable for its fruits and that nurseries were no longer interested in it, but he made no mention of infectiousness of the variegation. Leroy's training and interests seem to warrant the inference that had he noted the occurrence he would have recorded it. If the variegation in Leroy's trees was not infectious, Noisette's trees of the same variety manifested the not impossible but highly improbable coincidence of infectious variegation with a noninfectious variegation. The probability is, therefore, very strong that Leroy cultivated infection-carrying trees without noting the infectious nature of the variegation. Others may have done the same thing.

METHODS

In the spring of 1928 the Department of Horticulture of the Michigan Agricultural Experiment Station began an experiment designed to determine whether the practice, rather common among nurserymen, of working certain weak-growing varieties of pear and apple on vigorous intermediate varieties has any effect beyond making a good nursery tree. For stocks, 2-year-old apple seedlings, raised at East Lansing in 1926 and lined out for one year, were used. These were grafted close to the ground (but high enough to preclude scion rooting) with scions of the Red Canada (Steele Red [6]) apple and of other varieties designed to serve as intermediates to Steele. The Steele scions were secured from an orchard at Ovid, Mich., where they were grown as top grafts on Tolman Sweet. The further history of this strain is not known beyond the fact that the scions were secured by the late T. A. Farrand from an orchard which he described as the best Steele orchard in Michigan. No other Steele grafts or buds were used in the nursery until 1931.

During the growing season of either 1928 or 1929, small spots, some yellow and some cream white, of irregular outline (fig. 1), were noted on some leaves of the Steele grafts, but no significance was attached to them, and the work of building double-worked trees went ahead in 1929 and 1930, according to the original plan. When the leaves opened in 1930, a seedling which had been unsuccessfully grafted with Steele in 1928 showed yellow spotting in a very pronounced degree. Further examination revealed several other seedlings similarly affected; in all cases, however, they were seedlings which had been grafted to Steele, unsuccessfully. Variegation was found on a considerable number of living Steele scions, and in a few—not all—cases where seedling sprouts had not been completely eliminated variegation was found on their leaves. On a few trees which had been root-grafted in 1927 and top-grafted to Steele, with scions of the same origin, variegation was found in Steele leaves, in leaves of the Nixonite intermediate, but not in leaves of Virginia Crab used as an intermediate. Careful scrutiny of every tree in the nursery revealed no variegation in any tree except some of those which had been grafted, successfully or unsuccessfully, to Steele.

The manifestly infectious nature of this variegation raised two questions: (1) Whether the disease is endemic in the Steele variety and (2) whether other varieties are resistant or immune.

[6] In Michigan two distinct strains of Red Canada have been recognized, and one has been designated Steele, the name used in this paper. The material used was supposedly of the Steele strain.

The belief that the disease might be endemic was strengthened by vague reports that this variegation is more or less characteristic of Steele in the nursery. Though an inspection of all trees of this variety in two large nurseries in June, 1930, revealed no trace of variegation, the question was not regarded as definitely settled inasmuch as variegation had been found on seedling sprouts of trees whose Steele leaves showed no trace of it, and this seedling index was unavailable in most of the nursery trees. In addition, the usual slow growth of Steele trees and the common lack of vigor in variegated plants seemed to lend some support to this view. Accordingly, in the spring of

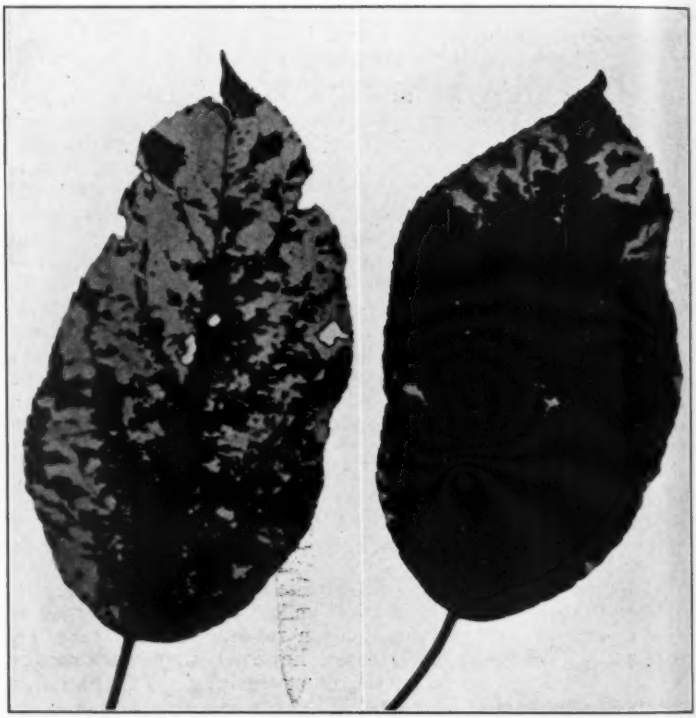

FIGURE 1.—Apple leaves showing infectious variegations

1931, scions from two other orchards, as well as those tracing to the original diseased strain, were used. Grafts of several other varieties were set on seedlings, high enough to permit retention of seedling leaves as indicators of transmission. These were budded in the ensuing August, record being kept of the source of buds in each case. With the unfolding of the leaves in 1932, the effects of these various manipulations could be appraised.

In 1930 variegation was recorded by the senior author; in 1931 and 1932 by the junior author. Since differentiation between very small spots due to variegation and others due to insects is a rather nice matter, the junior author made his diagnosis in ignorance of the

history of the trees, which were marked only by jumbled numbers. This removal of personal bias makes some of the findings particularly striking. Variegation was arbitrarily recorded as "trace", "moderate", and "heavy." Though no rigid standard was maintained, classification was made on the basis of conspicuousness, which, analyzed, signifies the development of large variegated areas rather than number of leaves affected.

RESULTS OF EXPERIMENTS

Three-year records (1930–1932) are available for 109 trees of Steele worked directly on seedlings. Of the 40 which showed variegation in the first year, 33 have shown it in the other two. Of the 69 which showed no variegation in 1930, 47 showed it in 1931; of the remainder, 11 showed it in 1932. Only 11 have shown no variegation in any of the three years. It is possible that some of these are free from variegation, since four buds from one of them set in Fameuse grafts in 1931 failed to produce variegation, a sequence not equaled in any budding from trees obviously infected. The increase in percentage of trees affected from 1930 to 1931 is not interpreted as evidence of the spread of the disease from tree to tree, for trees not grafted to Steele, although intermingled freely with those that were grafted to Steele, in no case developed the disease.

In these first trees propagated before variegation was recognized, efforts were made to prevent development of sprouts from the seedling stocks. Consequently data on transmission to the stocks must be drawn from observations on generally very reduced sprouts on 40, 35, and 26 trees in the respective years. The small number of growing points on these sprouts obviously reduced the chances of observation. In those cases in which observation was possible, however, variegation was found in 15 per cent in 1930, 34 per cent in 1931, and 19 per cent in 1932.

More reliable figures on transmission to seedlings are available from 93 grafts of the same strain set higher on seedlings in May, 1931. In June, 1932, 63 per cent of these scions and 46 per cent of their stocks showed variegation. No explanation is offered for the smaller percentage of variegation on the older trees in 1932 beyond the possible effect of transplanting on a portion of them. Those that were dug in the fall of 1931 and reset in the spring of 1932 showed much less variegation in the following summer.

Steele scions from another orchard were set in the spring of 1931. Of these, 109 were set on seedlings and 60 top-worked into various varieties, and in the following August 58 buds from these scions were set. In all, then, 257 opportunities were afforded this lot to show variegation; actually, 1 case was found. Accounting for this single case is not attempted; a mixture of scions is possible, since on one or two occasions the operator carried two lots at the same time. Nine scions from a third orchard failed to develop any visible variegation or to transmit it to young seedlings, and 112 of them top-worked into a mature tree failed to develop any symptom of variegation. Apparently, then, infectious variegation is not universal in this variety. On the other hand, buds taken from a mature Steele tree in the college orchard in 1931 developed variegation in stocks on which they were worked, and in shoots developing them, though examination of this tree in 1930, 1931, and 1932 revealed no variegation.

The best comparable data available on varietal susceptibility is that afforded by the grafts and buds set in 1931, as recorded in June, 1932. These are presented in Table 1. These percentages would probably be greater if the experiment were continued another year, but it appears at present that Tolman Sweet and Northern Spy are much less inclined to show variegation than the other varieties. Not only is the percentage of visibly affected trees smaller, but the amount of variegation as measured by percentage of leaves and area per leaf involved is distinctly lower.

TABLE 1.—*Appearance in 1932 of infections variegation in apple trees of several varieties top-worked with infected Steele scions in 1931*

Intermediate variety	Trees observed	Variegation shown in—		
		Steele scion	Intermediate	Seedling stock
	Number	*Per cent*	*Per cent*	*Per cent*
None (worked direct)	93	63	----------	46
Tolman Sweet	44	57	9	9
Wolf River	19	95	47	44
Northwestern Greening	14	100	79	21
Fameuse	69	64	42	22
Delicious	17	77	53	(a)
Yellow Bellflower	10	50	40	(b)
Oldenburg (Duchess)	9	100	38	38
Northern Spy	16	56	0	13
Virginia Crab	26	71	46	(a)

[a] Root-grafted.
[b] No observations possible.

The danger of attaching significance to one year's results is illustrated by the Virginia Crab. Eight nursery trees of this variety were top-worked in 1928 with Steele scions which subsequently proved to be variegated; others were top-worked with similar scions in 1930 and 1931. Variegation appeared in the Steele scions rather consistently in 1930 and 1931, but none was found in Virginia Crab, and until the leaves unfolded in 1932 this variety was regarded as immune. In these earlier propagations comprising 35 trees the sole case of variegation in Virginia Crab occurred in 1932. The figures presented in Table 1 represent a later propagation and present a totally different condition.

The lower percentage of seedling stocks showing variegation, as contrasted with the grafts set on them, has been attributed to the smaller number of leaves available for observation, rather than to resistance. This view is taken because of their more pronounced manifestation of variegation when it does occur. Without exception, the proportion of cases of pronounced variegation to all cases found was higher in seedling stocks than in the scions (Table 2). The small number of observations vitiates the results with Oldenburg, Northwestern Greening, and Northern Spy, but the consistency of the direction of the differences compensates in some measure for the paucity of material. Apart from this consideration, any attempt to differentiate between conditions which might be distinguished as susceptibility, manifestation of infection, tolerance, or resistance would be unwarranted on the basis of the evidence here available. Since varieties appear to differ, at least in their manifestation, seedlings probably do likewise. Consequently triple-worked trees, or

clonal rootstocks, would be necessary for further progress in this direction.

TABLE 2.—*Percentages of variegation cases classified as pronounced.*

Name of intermediate	Steele	Intermediate	Root
Direct	25		36
Fameuse	18	18	58
Tolman Sweet	25	0	50
Wolf River	22	11	88
Oldenburg	22	0	33
Northwestern Greening	6	8	33
Northern Spy	0	(a)	50
Combined	20	13	46

[a] No variegation recorded.

Transmission from stock to scion has occurred. Scions uninfected when set in 1931 into a mature tree, which in 1930 had shown a slight evidence of variegation, were rather heavily infected in 1932, though traces on the stock were still very inconspicuous. Variegated scions set in mature trees produce variegation which spreads throughout the tree, even to the tips of ungrafted limbs.

No indication of rapid spread of this disease from tree to tree has been observed. With the one exception already noted, no case has been found in the nursery which could not be traced to a graft, successful or unsuccessful, with infected scions. Infected and uninfected trees have been intermingled thoroughly, and no effort at insect control has been made. Spread through meeting and natural grafting of roots seems entirely possible, since sprouts originating on roots below ground have shown variegation, but no such case has been observed.

No opinion can be advanced here as to the effect of this disease. Two mature variegated trees are dying back at the tips of the branches; two others are distinctly subnormal, but since these trees have other troubles their weak condition can not be definitely attributed to variegation. In the nursery, variegated trees have shown no noticeable diminution of vigor.

Other cases showing identical symptoms have been observed. Two mature trees, one of the variety Marks and the other unidentified, in the college orchard have given clear, if inconspicuous, indication of the disease; a tree of Star has become infected by infected grafts, and a clon of root sprouts taken in 1926 from a tree since removed from the college orchard is thoroughly infected. Single cases occur in other parts of Michigan, involving mature Baldwin, Wolf River, and Fameuse trees; in one case variegated leaves were sent in as specimens of spray burn. Transmission from variegated Baldwin scions through a mature Wolf River tree to other Baldwin scions has occurred at the Graham Horticultural Experiment Station, Grand Rapids, Mich.

Attention is called to the apparent suitability of the apple as material for fundamental investigation of infectious variegation. The large number of clonal varieties with clear and well-recognized differences in vigor and responsiveness to environmental conditions, together with the general ease of handling the apple in the field and

in the laboratory, provide an unusually good foundation for pathological studies.

SUMMARY

Attention is called to the fact that one of the first cases of infectious variegation recorded was in the apple.

Varieties of apple differ, if not in susceptibility, at least in manifestation of infectious variegation.

None of the varieties tested is regarded as wholly immune.

Seedling stocks showed higher proportions of pronounced manifestations of this disease than did the varieties worked on them.

Buds taken from a tree showing no variegation produced it in seedling stocks.

Isolated apparent cases of variegation on several varieties are mentioned.

The effect of the disease on the tree has not yet been determined.

LITERATURE CITED

(1) Bossin.
1867. La panachure chez les végétaux. Rev. Hort. [Paris] 39: 452–453.

(2) Braun, K.
1929. Bericht über das auftreten von schädlingen und krankheiten im obst- und gemüsebau im regierungsbezirk stade während der monate juli und august 1928. Landwirtschaft Nos. 6 and 7, Wochenbeilage to Stader Tageblatt v. 8, no. 15.

(3) Cane, H.
1720. On the change of colour in grapes and jessamine. Roy Soc. [London], Phil. Trans., Ser. B. 31(366): 102. Also in Roy. Soc. [London], Phil. Trans., Ser. B. abridged ed. (1713–1723) 6: 489–490, 1809.

(4) Carrière, E. A., and André, E.
1889. Pommier franc à feuillage panaché. Rev. Hort. [Paris] 61: 6.

(5) Clinton, G. P.
1915. Chlorosis of plants with special reference to calico of tobacco. Conn. Agr. Expt. Sta. Ann. Rpt. 1914, pt. 6, p. [357]–424.

(6) Duchartre, P.
1870. Inoculation de la panachure par la greffe; exposé historique. Belg. Hort. 20: 113–118.

(7) Leroy, A.
1867–79. Dictionnaire de pomologie, contenant l'histoire, la description, la figure des fruits anciens et des fruits modernes les plus généralement connus et cultivés, 6 v., illus. Paris.

(8) Morren, E.
1870. Contagion de la panachure (variegatio). Belg. Hort. 20: 14–19, illus.

(9) Morse, W. J.
1916. Two apple-leaf troubles new to Maine. Maine Agr. Expt. Sta. Bul. 252: 186–190.

(10) Noisette, L. [C.]
1825–26. Manual complet du jardinier maraicher, pepiniériste, botaniste, fleuriste et paysagiste. 4 v., illus. Paris.

(11) Sageret, [A.]
1830. Pomologie physiologique. Paris. p. 42.

(12) Stewart, F. C.
1910. Notes on New York plant diseases, I. N. Y. State Agr. Expt. Sta. Bul. 328, p. [305]–404, illus.

(13) Vibert.
1863. Observations relatives a l'influence qu'exerce la greffe sur le sujet. Jour. Soc. Imp. et Cent. Hort. [France] 9: 144–145.

EXPERIMENTS ON THE CONTROL OF SEED-BORNE DISEASES BY X RAYS[1]

By W. R. TASCHER

Assistant Professor, Department of Field Crops, Missouri Agricultural Experiment Station

INTRODUCTION

Seed-borne diseases are those whose causal organisms are carried either within, or upon the surface of, the dormant seeds. The plants from infected seeds may develop serious seedling infections or, in case the disease is systemic, disorders in the mature plants. There are a number of crop seeds which carry the causal organisms of economically important diseases.

The losses resulting from seed-borne diseases vary, depending on the areas under consideration and the care used in seed selection. In some cases they have been estimated at as much as 10 per cent of the crop (*2*).[2] Among the organisms that infect corn, four were selected for the purpose of this study: *Diplodia zeae* (Schw.) Lév., *Gibberella saubinetii* (Mont.) sacc., *Fusarium moniliforme* Sheld., and *Cephalosporium acremonium* Cda. Of these *D. zeae* and *G. saubinetii* are probably the most serious, especially from the standpoint of corn-seedling injury. Other organisms studied were loose smut, *Ustilago tritici* (Pers.) Rostr., and bunt, *Tilletia levis* Kühn, of wheat; loose smut, *U. nuda* (Jens.) Kell. and Sw., of barley; and loose smut *U. avenae* (Pers.) Jens., of oats.

The recommended control of these organisms includes the application of germicides to the seed surface, and temperature treatments. Carefully conducted tests have indicated that organic mercury compounds are valauble in the control of certain organisms attacking corn. Copper carbonate and formaldehyde are widely used in the control of certain cereal diseases, and hot-water treatments have been used successfully in controlling loose smut of wheat.

The problem of controlling seed-borne organisms with X rays involves a study of the host reaction as well as that of the parasite. The X-ray treatment should not injure the host. Additional practical considerations are the efficiency of this treatment as compared with others and the cost. The use of X rays would permit treatment at any time before planting and would not unfit the seed for food purposes as do many chemicals.

REVIEW OF LITERATURE

The results obtained by using X rays as the sterilizing agent have varied. Much work has been done on the treatment of bacteria, especially those parasitic on man. Only those references dealing with the irradiation of plants will be cited.

[1] Received for publication Aug. 9, 1932; issued June, 1933. Contribution from the Department of Field Crops, Agricultural Experiment Station, University of Missouri, Journal Series No. 344. A part of a thesis presented in partial fulfillment of the requirements for the degree of doctor of philosophy under the direction of Dr. L. J. Stadler.

[2] Reference is made by number (italic) to Literature Cited, p. 914.

Trumbull and Hotson (*12*) tried X rays as a means of controlling *Fomes pinicola* (Fr.) Cke. on logs of fir and western hemlock, but without success. Levin and Levine (*5*) inoculated a series of castor beans, *Ricinus communis*, with *Bacterium tumefaciens* EFS. and Town. and immediately afterwards subjected the inoculation site to X rays. Six treatments given at intervals of two days prevented the development of tumors, apparently with no injury to the host plants. Rivera (*9*) described the successful control of *Bact. tumefaciens* tumors on castor-beans and geranium plants. In a later paper (*11*) he concluded that the preventive action was a function of the effect of radiation on the host tissue rather than on the bacterial cells. Nadson and Philippov (*7*) found the vegetative tissue of Mucor more resistant to X rays than were the sporangia. Small doses appeared to stimulate the development of *M. genevensis*.

Rivera (*10*) irradiated cereal seeds heavily infected by smut fungi. The treated cereals produced 36.1 per cent infected spikelets, and the untreated produced 36 per cent. Pichler and Wober (*8*) obtained favorable control results with spores of *Tilletia tritici* (Bjerk.) Wint. The killing action was augmented by increasing the acidity of the medium in which the spores were irradiated, particularly in the presence of oxygen or oxygen-yielding substances. Irradiated seed appeared to be injured slightly by the treatments, but potato tubers treated for the control of *Chrysophlyctis endobiotica* Schilb. were not injured. Preliminary observations by Mulvania (*6*) showed that X-ray treatments on the virus of tobacco mosaic had no apparent effect. Johnson (*4*) found that *Sclerotium bataticola* Taub., *Collybia dryophila* Fr., and *Fusarium batatatis* Wr. were unaffected by X-ray treatments.

EXPERIMENTAL PROCEDURE

In the study reported in this paper two phases of the problem of controlling seed-borne diseases by X rays were considered—the reaction of pure cultures of the fungi, and the reaction of the host and associated parasite to X rays.

The pure cultures were obtained from the botany department of the University of Missouri, with the exception of *Diplodia zeae* which was isolated from a corn seed.

Streak transfers were made to thin layers of potato-dextrose agar in Petri dishes. The cultures were incubated from three to five days before irradiation. Immediately before irradiation the colonies in each Petri dish were cut into small squares (3 to 4 mm). During irradiation the Petri dish covers were removed and replaced by a washed photographic film. The four fungi, each in a separate Petri dish, were irradiated simultaneously. After treatment the squares were removed from the culture and transferred to a Petri dish containing potato-dextrose agar. The effects of irradiation were noted after incubating the squares eight days at 28° C.

An old model Kelly-Koett X-ray machine was used. Intensity measurements were made with a Victoreen iontoquantimeter. A description of the dose given in each treatment includes the peak voltage, milliamperage, distance of the target from the material being X rayed, the length of exposure, and the dose in International r units per minute.

Lots of corn seed infected with *Diplodia zeae* and *Gibberella saubinetii* and nearly disease-free seed were obtained from Dr. J. R.

Holbert, Bloomington, Ill. The corn seeds for the isolation experiments were treated, germ side up, in a layer one seed deep. Those for the field experiments were exposed at random in a layer approximately three seeds deep.

The presence of internal parasites in the corn seeds was investigated by germinating the seeds on standard potato-dextrose agar at 28° C. The method used was similar to that of Chen (*1*).

The field plantings were in a fertile silt loam. The rows were 30 inches apart and the seeds 6 inches apart in the row.

The infected wheat, barley, and oats were secured from the Missouri experiment station.

TREATMENT OF PURE CULTURES OF FUNGI

Preliminary experiments with pure cultures of *Penicillium* sp., *Aspergillus* sp. and *Diplodia zeae* showed that exposure to X rays decreased the rate of growth of the fungi and that large doses caused death or inactivation. However, the doses required to kill the fungi in pure cultures seriously injured dormant corn seeds.

Cultures of *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and *Cephalosporium acremonium* were X rayed at different temperatures in order to establish their lethal doses and to determine the effect of temperature on the lethal doses. The cultures were exposed to the respective temperatures for 10 minutes preceding the treatment and during the period of treatment. Table 1 summarizes the effects of X rays, on the mycelium and spores of fungi at various temperatures.

TABLE 1.—*Effect of X rays on mycelium and spores of fungi in culture at various temperatures as determined by the development of X-rayed transfers*

[120 k. v. (peak), 4 ma., 16 cm target distance; 375 r units each minute]

Temperature (C°)	Fungus	Development after being X rayed for indicated period in minutes [a]									
		0	30	40	50	60	70	90	100	110	130
10	*Diplodia zeae*	+					+	+	+	+	+
	Gibberella saubinetii	+					+	+	+	+	+
	Fusarium moniliforme	+					+	+	+	+	+
	Cephalosporium acremonium	+					+	+	+	+	+
20	*Diplodia zeae*	+					+	−	−	−	−
	Gibberella saubinetii	+					+	+	+	+	+
	Fusarium moniliforme	+					+	+	+	+	+
	Cephalosporium acremonium	+					+	+	+	+	+
30	*Diplodia zeae*	+					−	−	−	−	−
	Gibberella saubinetii	+					+	+	+	+	+
	Fusarium moniliforme	+					+	+	+	+	+
	Cephalosporium acremonium	+					+	+	+	+	+
40	*Diplodia zeae*	+		−	−	−	−	−			
	Gibberella saubinetii	+		−	−	−	+	−			
	Fusarium moniliforme	+		+	+	+	+	+			
	Cephalosporium acremonium	+	−	+	+	+	+	+			
50	*Diplodia zeae*	+	−	−	−	−					
	Gibberella saubinetii	+	+	+	−	−					
	Fusarium moniliforme	+	+	+	+	+					
	Cephalosporium acremonium	+	−	−	−	−					

[a] Plus sign indicates growth; minus sign no growth.

The resistance of *Fusarium moniliforme* and the susceptibility of *Diplodia zeae* indicate a wide range in the tolerance of different fungi. The order of susceptibility to X rays was *D. zeae*, *Gibberella saubinetii*, *Cephalosporium acremonium*, and *F. moniliforme*.

The susceptibility of the fungi to X rays was increased by exposure to high temperatures. Cultures of the fungi used showed no pronounced effects of exposure to the temperatures alone. It is probable that the cultures X rayed at the extreme temperatures were not at the designated temperature throughout the X ray period because of the short pretreatment exposure of 10 minutes.

Diplodia zeae, when X rayed at 10° C. resumed growth after several days. After three days' incubation only a trace of mycelial development was noted in each of the cultures. Two days later large colonies had developed in those receiving 70 and 90 minute treatments, but there was little or no further development in the remaining cultures. Five days later the latter showed definite growth.

Of the cultures X rayed at 20° C. only the culture receiving the 70-minute treatment resumed growth at the end of the 10-day observational period.

The fungi used grew as well on X-rayed agar as on the unirradiated in these tests. However, it is possible that the X-ray treatment affected the medium on which the cultures had been growing, thus accounting wholly or in part for growth differences.

Diplodia zeae did not develop spores after a 4-day incubation at 28° C., while the remaining three fungi produced fruiting bodies to some extent. Chlamydospores were produced by *Gibberella saubinetii*. The presence of occasional resistant spores in these cultures may have accounted for the growth in the culture receiving the 70-minute treatment at 40°.

TREATMENT OF INFECTED CORN SEEDS

Corn seeds infected by *Diplodia zeae* and *Gibberella saubinetii* were used, since these fungi are most susceptible to X rays in pure cultures and are of more economic importance.

X-ray treatments (120 k. v. (peak), 4 ma., 16 cm target distance, 80 minutes, 375 r units per minute, 50° C. temperature) in the dormant stage of infected seeds did not kill the seed-borne fungi as shown by the vegetative growth made on potato-dextrose agar. This dose injured the corn seeds severely. The fungi in pure cultures are more susceptible than those in the dormant host. This is probably on account of the inactive and resistant form of the parasite and the partial absorption of the X rays by the seeds.

Treatments designed to increase the susceptibility of the parasite without increasing that of the host were attempted. Dormant corn seeds were exposed three days to air saturated with water vapor at 30° C. The control of *Diplodia zeae* was not effected by an X-ray dose distinctly injurious to the host. Corn seeds soaked for periods of 15, 30, 60, 120, and 240 minutes were not freed of *D. zeae* by an X-ray treatment suffcient to cause injury to the seed. Increasing the moisture content of the corn seeds to 30 per cent did not markedly increase the susceptibility of the parasite. The degree of infection resulting from *D. zeae* appears to decrease to some extent in X-rayed seed corn, with storage.

FIELD TESTS

In order to test further the effects of X rays on seed-borne organisms dormant corn seeds infected with *Diplodia zeae* and *Gibberella saubinetii* were X-rayed and planted immediately in the field. Seeds that had received an organic mercury treatment, as recommended

by Holbert, Reddy, and Koehler (*3*), were also planted in order to compare the effects of the two methods of treatment.

Forty kernels were used in each experiment and were planted 1 foot apart in the rows. Four plantings were made at weekly intervals. Infected seedlings were identified by examination of the roots and mesocotyls.

Control of the seed-borne diseases was not effected by the range of treatments used. Total doses of from 375 to 12,000 r units showed no effect except a definite decrease in plant growth when the larger doses were used. X rays having a range of wave lengths obtained by voltages of 56, 78, 120, and 120 k. v. filtered through 1 mm of copper had no effect on the percentage of infection. Intermittent treatments given at weekly intervals did not add to the preventive action. The organic mercury treatment decreased *Diplodia zeae* infection approximately 50 per cent but had no effect on *Gibberella saubinetii*. There was no evidence of stimulation in any of the treatments. The diseased plants were distinctly inferior to those produced by the nearly disease-free corn.

TREATMENT OF INFECTED CEREAL SEEDS

Wheat seeds infected with *Ustilago tritici* were X rayed (107 k. v. (peak), 4 ma., 16 cm target distance, 370 r units each minute, seeds 1 cm deep) in the dormant and germinating stages. A treatment of 30 minutes in the dormant stage reduced the percentage of infection to approximately one-third that of the untreated seeds and noticeably injured plant growth.

Germinating wheat seeds (soaked 12 hours, aerated 12 hours) were X rayed 5 minutes (375 r units each minute). Complete control of the fungus resulted, but the plants received serious injury. A 2½-minute treatment decreased infection approximately 50 per cent and also injured the plants. An equivalent treatment, a ¼-mm copper filter being used, reduced infection to approximately one-eighth that of the check without serious injury to the plants.

Dormant wheat seeds infected with *Tilletia levis* were treated similarly. Infected heads were observed in the plants that had received the 40-minute treatment. The percentage of infection of the untreated lot was too small to determine the extent of control.

An X-ray treatment (140 k. v. peak), 4 ma, 24 cm target distance, 240 r units each minute, seeds 1 cm deep) of dormant barley seeds for 68 minutes slightly reduced the infection of *Ustilago nuda* and noticeably reduced the stand and vigor of the plants. A 16-minute treatment of germinating barley seeds (soaked 8 hours, aerated 12 hours) effected complete control but reduced the stand to less than 5 per cent that of the untreated seeds.

X-ray treatments applied to oats in the germinating stage were more effective in the control of *Ustilago avenae* than were those applied to the dormant oat seeds. Doses (140 k.v. (peak), 4 ma., 24 cm target distance, 240 r units each minute, seeds 1 cm deep) applied for 17 minutes to dormant seeds reduced infection approximately 50 per cent. An X-ray treatment of 68 minutes did not reduce infection further. Infection was reduced approximately 25 per cent in the oats treated in the germinating stage (soaked 4 hours, aerated 4 hours) for 33 minutes, but the mature oat plants were slightly injured. This suggests that some of the organisms are more susceptible to X rays.

These treatments indicate that the differentials used in the killing points of the hosts and parasites are not large enough to allow control without serious injury to the hosts. Germinating the seeds affected the differential favorably. The complete control effected in the case of loose smut of barley suggests the use of X rays in investigations where chemical residues are not desired, and where a fraction of the original host population is sufficient.

Negative results secured by other workers, where comparisons could be made, may be ascribed to too short a period of treatment with X rays.

SUMMARY

The control of seed-borne diseases by X rays depends on the differential in the killing points of the hosts and their parasites. This differential was found not to be sufficient to permit the complete control of the organisms studied.

While *Diplodia zeae* was quite susceptible to X rays in the actively growing cultures, it was extremely resistant in the dormant corn seed. This probably is due to the inactive condition of the organism, the presence of resistant spores, and the absorption of radiant energy by the seeds. An X-ray dose several times the lethal dose for corn was required to affect the organism.

The decreasing order of tolerance of the fungi used in culture was: *Fusarium moniliforme*, *Cephalosporium acremonium*, *Gibberella saubinetii*, and *Diplodia zeae*. The possible presence of spores in all but *D. zeae* at the time the fungi were X rayed may account for their greater resistance.

Supplementary treatments used in an attempt to modify the fungus or host in order to obtain a more favorable difference in killing points were not successful. The treatments affected the host and parasite similarly.

The organic mercury treatment was effective in partially controlling *Diplodia zeae* but appeared to be ineffective with *Gibberella saubinetti*.

X-ray treatments of dormant wheat, barley, and oat seeds infected with *Ustilago tritici*, *Tilletia levis*, *Ustilago nuda*, and *U. avenae*, respectively, did not effect control.

X-ray treatments of germinating seeds significantly decreased the percentage of loose smut of oats and completely controlled loose smut of barley, although germination was decreased to as low as 5 per cent in the barley.

Seeds as well as fungi vary widely in their lethal X-ray doses. It is possible that seeds requiring large lethal doses may be infected by fungi requiring small lethal doses, in which cases control by X rays may be effective.

LITERATURE CITED

(1) Chen, C. C.
1920. internal fungous parasites of agricultural seeds. Md. Agr. Expt. Sta. Bul. 240, p. [81]–110, illus.

(2) Holbert, J. R., Burlison, W. L., Koehler, B., Woodworth, C. M., and Dungan, G.
1924. corn root, stalk, and ear rot diseases, and their control thru seed selection and breeding. Ill. Agr. Expt. Sta. Bul. 255, p. 239–478, illus.

(3) ———, Reddy, C. S., and Koehler, B.
1928. chemical-dust seed treatments for dent corn. U. S. Dept. Agr. Circ. 34, 6 p.

(4) JOHNSON, F. H.
1932. EFFECTS OF ELECTROMAGNETIC WAVES ON FUNGI. Phytopathology 22: 277–300, illus.
(5) LEVIN, I., and LEVINE, M.
1917. THE INFLUENCE OF X RAYS ON THE DEVELOPMENT OF CROWN GALL. Soc. Expt. Biol. and Med. Proc. 15: 24–25.
(6) MULVANIA, M.
1926. STUDIES ON THE NATURE OF THE VIRUS OF TOBACCO MOSAIC. Phytopathology 16: 853–871.
(7) NADSON, G. A., and PHILIPPOV, G. S.
1925. INFLUENCE DES RAYONS X SUR LA SEXUALITÉ ET LA FORMATION DES MUTANTES CHEZ LES CHAMPIGNONS INFÉRIEURS (MUCORINÉES). Compt. Rend. Soc. Biol. [Paris] 93: 473–475.
(8) PICHLER, F., and WÖBER, A.
1922. BESTRAHLUNGSVERSUCHE MIT ULTRAVIOLETTEM LICHT, RÖNTGENSTRAHLEN UND RADIUM ZUR BEKÄMPFUNG VON PFLANZENKRANKHEITEN. Centbl. Bakt. [etc]. (II) 57: 319–327.
(9) RIVERA, V.
1926. AZIONE DEL RAGGI X SUPRA I TUMORI VEGETALI. Riv. Biol. 7: 449–465, illus.
(10) ———
1926. CARIE DEL FRUMENTO E RAGGI X. R. Staz. Patol. Veg. [Rome], Bol. Mens. (n. s.) 6: 237–241.
(11) ———
1928. AZIONOE DI FORTI DOSI RAGGI Y SOPRA IL "B. TUMEFACIENS" SMITH ET TOWNSEND. Atti R. Accad. Naz. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat. (6) 7: 867–869.
(12) TRUMBULL, H. L., and HOTSON, J. W.
1917. THE EFFECT OF ROENTGEN AND ULTRAVIOLET RAYS UPON FUNGI. Phytopathology 7: [426]–431, illus.

179377—33——4

EFFECT OF LONG-CONTINUED MUSCULAR EXERCISE UPON THE CHEMICAL COMPOSITION OF THE MUSCLES AND OTHER TISSUES OF BEEF CATTLE[1]

By H. H. MITCHELL, *Chief in Animal Nutrition*, and T. S. HAMILTON, *Associate in Animal Nutrition, Department of Animal Husbandry, Illinois Agricultural Experiment Station*[2]

INTRODUCTION

The quality and palatability of meat are dependent to a great extent upon the chemical and physical properties of the lean, which consists largely of muscle tissue. Among the factors affecting the quality of lean, therefore, muscular exercise may very well be of outstanding importance. Attempts to modify the composition of lean meat by varying the ration fed to the animal have not been successful, since it is evident that cellular protoplasm is capable of maintaining its composition in the face of a varying food supply. But variations in the intensity of functioning of muscle are known to occasion variations in the content of certain of its constituents.

Many investigations have been concerned with a study of the effect of contraction upon the composition of muscle, and much light, especially in recent years, has been thrown upon the problems of muscular metabolism and of the origin of muscular energy. But the ephemeral changes associated with contraction, relaxation, and recovery are not of direct importance to the question of the differences in the composition of meat in an animal accustomed to muscular exercise and in one unaccustomed to muscular exercise. The effects of muscular training on the composition of muscle tissue may be expected to be of a much more permanent nature.

REVIEW OF LITERATURE

Rogozinski (*24*)[3] found that the muscles of a dog that had been worked for 12 days contained less water and less fat than did those of a dog that had not been worked. However, the data obtained in an experiment with another pair of dogs did not support the conclusion that long-continued work causes a withdrawal of water. Gerhartz (*12*) experimenting with two pairs of dogs, confirmed the conclusion that muscular work decreases the water content of muscles. On the other hand, Embden and Habs (*8*) and Procter and Best (*22*) found no such effect of training.

The most consistent outcome of experiments concerned with the effect of long-continued muscular work on the chemical composition of muscle relates to the content of glycogen. Although the immediate effect of muscular contraction is to lower the glycogen stores, even

[1] Received for publication Nov. 28, 1932; issued June, 1933.

[2] The investigations described in this paper were performed in cooperation with the Beef Cattle Division and the Meat Division of the Illinois Agricultural Experiment Station and the Bureau of Animal Industry of the U. S. Department of Agriculture. Each of these groups undertook independent studies, the results of which will be reported elsewhere. The contribution of the Division of Animal Nutrition was the product of a team of workers, for whose efforts the authors here make due acknowledgment. The contributions of the following members of the staff and part-time workers deserve special mention: F. J. McClure, W. T. Haines, F. I. Nakamura, Frank Simpson, H. D. Preston, T. E. Aspinall, and Mrs. Harriette King Klabunde.

[3] Reference is made by number (italic) to Literature Cited, p. 939.

to almost complete exhaustion in the case of protracted work, the repeated performance of muscular work has been found, in many experiments, to increase markedly the glycogen content of muscle, as much as two or three times the normal value, according to Embden and Habs (*8*), who used rabbits trained for periods of 2 to 6 weeks. The high content of glycogen in the trained muscles disappears very gradually after the training period is terminated. Procter and Best (*22*) in their experiments on dogs found that training periods of 7 to 16 days induced a marked increase in glycogen storage in muscles, but that longer periods of training, 20 to 42 days, produced no distinct effect. These results suggested to the authors that there is an optimum period of training for the production of increased glycogen stores and hence that these induced excessive stores are not an essential concomitant of increased ability to perform muscular exercise.

The effect of muscular training on the creatine content of muscle has not been so clearly determined. The most comprehensive of recent investigations concerned with this question are those of Palladin and Ferdmann (*21*), who experimented with rabbits. Muscular stimulation was brought about by an induction current, applied over the biceps femoris of one leg, the biceps femoris of the other leg serving as a control. In the first experiment, two rabbits were killed after a 5-minute period of stimulation, and the two biceps femoris analyzed. In both rabbits, the stimulated muscle was greatly impoverished in glycogen and contained approximately 4.5 per cent more water than did the unstimulated. The percentage of creatine on the fresh basis was lower in the stimulated muscle than in its control, but expressed either on the dry basis or as a percentage of the total nitrogen content, the percentage of creatine was very nearly the same in stimulated and unstimulated muscle. An entirely different result was obtained, however, when repeated stimulation to induce a condition of muscular training was resorted to. When a 3-minute stimulation was repeated every 4 hours for periods of 5 to 15 days and when the rabbits were sacrificed for chemical examination 48 hours after the last stimulation, the creatine content of the trained biceps femoris was markedly increased over that of the untrained in eight experimental animals. The glycogen content was increased also, while in two of the rabbits on which determinations are reported, there was no difference in moisture content. One or two days training was not sufficient to bring about these results, and it was also shown, in other rabbits, that the effect on the creatine content of the muscle had largely disappeared in 2 or 3 days and entirely in 6 days. The increased glycogen content was plainly evident after six days.

In apparent contradiction to these excellent and consistent experiments of Palladin and Ferdmann may be cited the earlier negative experiments of Mellanby (*18*) on isolated frog muscle and rabbit muscle in situ, and of Brown and Cathcart (*5*) on decerebrated frogs and later on rabbits. In fact, in the latter case stimulation appeared to have depressed the creatine content of muscle. However, these experiments did not involve the training of muscle, but rather short-time stimulation. In the experiments of Embden and Habs (*8*) no change in the creatine content of the trained muscle was observed in two rabbits, while in two other rabbits what appeared to be a significant depression in creatine content was found. On the other hand, Ferdmann and Feinschmidt (*9*), using much the same pro-

cedures as Palladin and Ferdmann and the same species of animal, were able to show that, under their conditions, training increased the creatine-phosphoric acid content of muscle and that this increase disappeared in the course of six days of muscular rest. In these experiments the hexose-phosphoric acid and the pyrophosphoric acid of muscle were not affected by muscular training.

These investigations on the effect of muscular work upon the composition of muscle indicate (1) that the first effects of work are related to the reactions occurring during contraction and recovery, from which the energy is derived; (2) that continued work, or muscular training, produces changes in composition, such as an increase in the content of glycogen and of creatine, either the direct reverse of those related to the contraction phase, or unrelated to them; and (3) that protracted training may cause the first effects of training to disappear gradually and even may cause contrary effects to appear, such as a decrease in creatine content.

The experiments reported in this paper relate to the effects of long-continued muscular training.

PLAN OF THE EXPERIMENTS

The experiments extended over two years, from February, 1929, to May, 1931. In each experiment, the subjects were eight steer calves of predominantly Hereford blood, obtained from the same locality and of the same breeding and previous treatment. They weighed initially from slightly less than 500 pounds to slightly more than 600 pounds, and in each experiment they were fairly uniform, especially in the second year's work. The experimental treatment continued in the first experiment from February 13 to June 15, a period of 122 days, and in the second from January 7 to May 18, a period of 131 days.

In the first experiment,[4] the eight steers were divided into two lots of four each, one lot being exercised daily, the other lot being closely confined. The exercised lot were worked individually for one hour each day on a treadmill set at a slight angle, and the work continued up to the day before slaughter. Each steer walked the equivalent of 450 miles on the horizontal plane, an average of 3.6 miles per day. The exercised steers were full fed a ration of shelled corn, cottonseed meal, corn silage, and alfalfa hay. The unexercised lot were fed the same ration, but in amounts limited so that their gains and conditions would approximate those of the exercised steers. These steers were confined in a small pen in a feeding shed open to the east. The exercised steers also were kept in this pen except during the exercise period, when they were removed to a treadmill next to the pen.

The slaughter weights of the exercised steers averaged 833 pounds, and those of the unexercised steers, 826 pounds. The average daily gains in the two lots were, respectively, 2.33 and 2.56 pounds.

At the time of slaughter, samples of blood for examination and analysis were taken from each steer. Also a special sample of muscle tissue was taken from the upper and inner round for immediate analysis. The livers and hearts (trimmed) were also taken for analysis. From the chilled carcasses, the total lean from each of the nine wholesale cuts from one-half of the carcass, separated from all overlying fat by knife, was ground and sampled for chemical analysis.

[4] For reasons explained below, this experiment is hereafter designated as experiment 2.

The plan of the second experiment [5] was the same as that of the first, with three exceptions. The steer calves were paired as to initial weight and grade, one calf of each pair being exercised, and during the experimental period the weights of each pair were kept approximately the same by restricting the food consumption of the unexercised calf, the other calf having unrestricted access to food. The ration fed was the same as in the preceding experiment, but the exercise imposed was much greater. Starting at one hour's exercise daily, the period of work was increased to approximately three and a half hours daily. Each exercised steer walked the equivalent of 1,181 miles in 381 working hours, amounting to 8.8 miles per day at a rate of 3.1 miles per hour. As in the preceding experiment, the work was continued up to the day of slaughter. At the time of slaughter, the same samples were taken as in the first experiment, but the selection of lean samples from the chilled carcasses was different, being confined to certain selected muscles from a number of the wholesale cuts of one-half of each carcass. The muscles and the cuts from which they were taken are as follows:

(1) From the round, taken from the rump to the stifle joint, the following muscles were separated: The semimembranosus and adductor muscles, the semitendinosus muscle, and the biceps femoris muscle.

(2) From the short loin, the longissimus dorsi and psoas major muscles were taken.

(3) From the sixth to the twelfth ribs, inclusive, the longissimus dorsi was removed.

(4) From the flank cut, the obliquus externus abdominis was taken.

(5) From the chuck, the triceps brachii muscle was removed from the head of the humerus to the elbow.

The slaughter weights of the unexercised steers in the second experiment averaged 751 pounds, and those of the exercised steers 729 pounds. The average daily gains were, respectively, 1.70 and 1.57 pounds.

In the samples taken, moisture was determined by drying in an electric oven at 105° C. for 5 hours, fat by continuous extraction of the dried samples with ether for 48 hours, nitrogen by the Kjeldahl method, using mercury and potassium sulphate in the digestion mixture and distilling into 4 per cent boric acid, and ash by ignition in an electric muffle furnace at temperatures below 700°. Iron was determined by a slight modification of the Kennedy (*14*) method, hemoglobin by a modification of Whipple's method (*31*), and creatine by the procedure developed by Rose, Helmer, and Chanutin (*25*). The collagen and elastin determinations were made by a method developed in this laboratory (*19*).

RESULTS OF THE EXPERIMENT

Although the experiment involving the greater degree of muscular exercise was performed after the experiment involving the smaller degree, it seems advisable to consider it first in the discussion of the results obtained, because the effects of training would be the more pronounced and the more easily discerned. Therefore, in the following discussion it will be referred to as experiment 1.

[5] For reasons explained below, this experiment is hereafter designated as experiment 1.

THE BLOOD

The hemoglobin in the blood was determined by the Newcomer acid hematin method (*20*), using a disk that had been standardized by the oxygen capacity method of Van Slyke and Stadie (*28*). The hemoglobin was determined in a fresh sample of blood, all other constituents in oxalated samples. The results of the determinations are summarized for both experiments in Table 1. There appears to be no effect of exercise on either the water content, the erythrocyte count, the hemoglobin content, or the iron content.

TABLE 1.—*Results of the examination and analysis of the blood samples from steers on heavy and on light work, and from unexercised steers*

Experiment 1, heavy work					Experiment 2, light work			
Treatment and steer No.	Water content	Red cell count	Hemoglobin	Iron content	Treatment and steer No.	Water content	Red cell count	Hemoglobin
	Per cent	*Millions per mm³*	*Grams per 100 cc*	*Per cent*		*Per cent*	*Millions per mm³*	*Grams per 100 cc*
Exercised:					Exercised:			
84	79.68	9.47	11.8	0.0413	80	79.12	7.73	13.7
9	81.12	8.55	10.6	.0349	116	80.41	6.06	12.5
62	80.26	10.58	11.6	.0433	119	78.77	7.22	14.3
34	79.72	8.95	12.2	.0457	125	78.93	10.60	13.1
Average	80.20	9.39	11.6	.0412	Average	79.31	7.90	13.4
Unexercised:					Unexercised:			
23	80.97	12.17	11.5	.0370	103	80.09	4.92	12.0
21	81.34	10.17	10.7	.0372	117	78.84	8.78	13.7
43	79.45	10.22	11.5	.0455	121	78.42	9.49	14.3
36	81.44	9.30	10.3	.0413	129	77.99	10.08	14.3
Average	80.80	10.47	11.0	.0402	Average	78.84	8.32	13.6

In both experiments, but particularly in experiment 1, the red cell counts are higher than those usually found for cattle blood. Thus, Marloff (*17*) has reported an average of 6.51 millions per cubic millimeter; Kuhl (*15*), 5.72; Wells and Sutton (*30*), 7.65; Schenkenbach (*26*), 8.05 for males and 7.47 for females; Bonnier, Jorpes, and Sköld (*4*), 5.46; Drastich (*7*), 6.41; and Hayden and Fish (*13*), 6.55.

The hemoglobin values in experiment 1, averaging 11.3 g per 100 cc, are approximately the same as the average of 11.7 reported by Marloff (*17*), 10.8 by Kuhl (*15*), 10.55 by Drastich (*7*), and 10.9 and 11.29 by McCay (*16*) in two large series of analyses of the blood of dairy cows. The latter investigators report an average of 12.2 ± 0.8 g of hemoglobin per 100 cc of blood for six mature bulls of four different breeds. The hemoglobin values in experiment 2, averaging 13.5 g per 100 cc of blood, although carried out by the same technic, were substantially higher.

The iron content of the blood, determined in experiment 1, averaged 0.0407 per cent. This value is somewhat higher than the average value obtained by McCay (*16*) of 38.9 mg of iron per 100 cc of blood, from 226 analyses, and the average value of 36.5 mg per 100 cc of blood reported by Awdejewa and his associates (*1*), from observations on nine cows. Wagenaar (*29*) obtained an average of 0.042 per cent of iron in the blood of four cows.

THE IRON AND HEMOGLOBIN CONTENTS OF THE TISSUES

Hemoglobin determinations were made upon the heart and the special sample from the round in experiment 1 (heavy work), and upon the liver, round, ribs, and shank samples in addition in experiment 2. The results of these analyses, expressed in percentage of the fresh tissue, are summarized in Table 2. They are of interest, in view of the scarcity of such information in the literature, even though they do not demonstrate any effect of muscular training. In experiment 2 (light work) the averages for five of the six samples are higher for the exercised than for the unexercised steers. However, the variations among the steers within each group were so great that average group differences are obviously insignificant.

TABLE 2.—*Hemoglobin content (per cent) of the tissues from steers on heavy and on light work, and from unexercised steers*

Experiment 1, heavy work			Experiment 2, light work						
	Hemoglobin in—			Hemoglobin in—					
Treatment and steer No.	Heart	Special sample of round	Treatment and steer No.	Liver	Heart	Special sample of round	Round	Ribs	Shank
Exercised:			Exercised:						
84	0.404	0.283	80	0.328	0.286	0.295	0.258	0.198	0.258
9	.382	.387	116	.352	.348	.225	.195	.196	.232
62	.325	.354	119	.405	.412	.211	.171	.162	.239
34	.463	.389	125	.297	.361	.260	.194	.174	.336
Average	.394	.353	Average	.346	.352	.248	.205	.183	.266
Unexercised:			Unexercised:						
23	.393	.388	103	.418	.338	.190	.170	.189	.257
21	.448	.370	117	.290	.325	.242	.208	.184	.210
43	.354	.346	121	.393	.292	.280	.181	.192	.192
36	.451	.361	129	.164	.264	.308	.170	.161	.253
Average	.412	.366	Average	.316	.305	.255	.182	.182	.228

In experiment 1 (heavy work) all of the eight samples of muscles taken from the chilled carcasses were analyzed for iron, making a total of 64 analyses. The results revealed no appreciable differences between cuts and no significant differences between exercised and unexercised groups. The average result was 0.003075 ± 0.000042 per cent of iron in the fresh tissues.

In experiment 2 (light work) iron analyses were made upon the marrow from one of the femur bones of each of the carcasses. On the same samples, analyses were made also for dry substance and fat. The results in Table 3 show that, in the group of exercised steers, the marrow contained 20 per cent less iron than did that of the unexercised group. By applying Fisher's modification of Student's method (*11, p. 107*) to these two groups of results, a value of $t = 4.83$ is obtained, and the probability that a difference as great as that observed (0.00052) would result from chance is considerably less than 0.01. This lowering of the iron content of the marrow was associated with an increased content of dry matter, but essentially no difference in fat content. There was an average of 4.14 per cent of dry matter not fat in the marrow samples from the exercised steers and an

average of only 1.94 per cent of dry matter not fat in the marrow samples from the unexercised steers.

TABLE 3.—*Chemical composition (per cent) of the marrow from the femur bones from steers on light work (experiment 2) and from unexercised steers*

Treatment and steer No.	Dry substance	Ether extract	Iron	Treatment and steer No.	Dry substance	Ether extract	Iron
Exercised:				Unexercised:			
80	97.43	95.23	0.00197	103	96.79	97.53	0.00237
116	97.88	94.62	.00216	117	96.71	93.58	.00282
119	95.58	89.32	.00206	121	91.24	89.45	.00256
125	97.41	92.58	.00214	129	95.21	91.63	.00266
Average	97.08	92.94	.00208	Average	94.99	93.05	.00260

THE FAT AND WATER CONTENTS OF THE TISSUES

The results of the analysis of the tissue samples for fat and water are given in Table 4. The fat percentages are on the fresh basis, but the water percentages are computed on the protoplasmic (fat-free) basis, in order to eliminate the considerable influence of variations in fat content.

TABLE 4.—*Fat and water content of tissue samples from steers on heavy and on light work, and from unexercised steers*

EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF FAT ON THE FRESH BASIS

Treatment and steer No.	Heart	Liver	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
				Semimembranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triceps brachii
Exercised:											
84	2.38	1.67	1.06	0.80	1.26	1.30	1.19	2.67	1.78	3.48	1.24
9	2.86	1.54	1.73	2.37	2.00	1.46	2.78	4.22	3.06	5.20	2.34
62	2.97	1.63	1.13	1.01	1.24	2.32	.96	2.26	1.68	2.06	1.97
34	2.88	1.56	1.28	1.38	1.68	1.39	2.20	3.08	2.14	2.77	1.83
Average	2.77	1.60	1.30	1.39	1.54	1.62	1.78	3.06	2.16	3.38	1.84
Unexercised:											
23	3.20	2.36	1.53	1.39	1.27	2.14	2.33	4.50	1.98	2.90	3.39
21	3.62	2.00	1.52	1.54	2.00	1.40	1.58	3.16	2.07	3.46	2.18
43	2.56	2.39	1.62	2.50	1.40	1.86	1.49	2.61	2.52	2.49	1.95
36	3.63	2.90	1.60	1.65	1.75	2.25	1.98	3.15	2.05	2.67	2.60
Average	3.25	2.41	1.57	1.77	1.60	1.91	1.84	3.36	2.16	2.88	2.54

EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF WATER ON THE PROTOPLASMIC BASIS

Exercised:											
84	80.60	71.01	75.73	76.13	76.07	76.40	75.85	77.54	76.91	76.86	76.54
9	80.21	71.52	75.71	75.65	76.20	76.20	76.32	76.99	76.34	75.79	76.51
62	81.08	71.20	76.31	76.13	76.39	76.48	75.92	77.95	76.88	76.49	77.32
34	81.11	70.94	76.60	76.11	76.05	76.57	76.23	77.87	76.79	76.08	77.58
Average	80.75	71.17	76.09	76.01	76.18	76.41	76.08	77.59	76.73	76.30	76.99
Unexercised:											
23	81.63	72.27	76.93	76.48	76.19	76.83	76.43	78.39	77.00	77.91	78.40
21	81.56	72.36	77.23	76.66	76.98	77.25	76.44	78.33	77.00	77.69	77.71
43	82.18	73.54	77.25	77.74	76.97	76.54	76.68	78.14	78.16	76.65	77.51
36	81.12	74.26	76.44	76.23	76.37	77.30	76.07	77.86	76.53	77.33	77.20
Average	81.62	73.11	76.96	76.78	76.63	76.98	76.40	78.18	77.17	77.39	77.70

TABLE 4.—*Fat and water content of tissue samples from steers on heavy and on light work, and from unexercised steers*

EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF FAT ON THE FRESH BASIS

Treatment and steer No.	Heart	Liver	Special sample of round	Round	Ribs	Fore shank	Loin end	Short loin	Rump	Plate	Chuck	Flank
Exercised:												
80	1.58	1.54	2.34	4.10	9.47	5.87	7.20	8.05	6.51	13.49	6.81	12.27
116	1.86	1.36	2.58	4.60	8.38	4.45	5.28	6.60	6.52	14.15	7.35	11.88
119	1.97	2.05	3.40	4.71	9.00	5.02	6.51	8.90	7.05	13.80	7.45	10.21
125	2.19	1.92	3.02	3.51	7.77	4.38	5.31	7.04	5.78	12.24	6.67	8.21
Average	1.90	1.72	2.84	4.23	8.66	4.93	6.08	7.65	6.47	13.42	7.07	10.64
Unexercised:												
103	(a)	2.68	4.13	5.14	12.13	4.67	8.06	10.46	9.43	14.93	8.69	(b)
117	2.55	2.72	2.34	4.37	8.94	5.15	5.31	8.26	5.29	13.35	6.93	9.80
121	2.17	2.54	2.51	2.20	8.81	3.99	6.19	7.07	5.29	10.54	6.99	9.94
129	2.14	2.57	2.27	3.62	9.45	5.96	5.28	6.82	6.47	12.96	7.23	8.00
Average	2.38	2.63	2.81	3.83	9.83	4.94	6.21	8.15	6.62	12.95	7.46	9.37

EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF WATER ON THE PROTOPLASMIC BASIS

Exercised:												
80	79.99	70.44	75.34	75.79	76.26	75.42	76.26	76.14	75.57	75.93	76.55	75.17
116	81.34	71.10	75.85	76.51	75.81	77.26	76.82	77.08	76.60	77.11	77.24	76.06
119	80.86	71.64	75.86	76.35	75.52	75.84	76.76	76.38	76.22	76.68	77.19	75.27
125	81.54	72.69	75.93	75.97	75.39	76.53	76.51	75.60	76.46	76.14	76.32	75.06
Average	80.93	71.47	75.74	76.15	75.74	76.26	76.59	76.30	76.21	76.46	76.82	75.39
Unexercised:												
103	81.85	73.62	76.25	76.13	77.28	77.24	77.49	76.67	76.32	76.11	77.09	(b)
117	80.99	73.55	75.34	76.97	77.26	76.59	76.82	76.35	77.53	77.03	77.09	75.85
121	81.04	74.31	76.07	76.26	76.68	76.61	76.27	77.67	76.04	76.07	76.70	75.69
129	81.23	73.27	75.05	75.67	76.27	75.94	76.15	75.86	76.56	76.47	76.73	75.05
Average	81.28	73.69	75.68	76.26	76.87	76.59	76.68	76.64	76.61	76.42	76.90	75.53

[a] The excess fat was not trimmed from this sample before analysis.
[b] This sample was lost.

In experiment 1 there is a general tendency for the tissues from the exercised steers to exhibit a smaller fat content than the corresponding tissues from the unexercised steers. Of the 11 tissues examined, only 2, the longissimus dorsi muscle of the rib cut and the obliquus externus abdominis of the flank cut, failed to show this relation between the averages of the two groups of steers.

The most pronounced difference in fat content occurred in the case of the liver samples. Here the average difference amounts to 0.81 per cent of fat; the value of t (essentially the ratio of the average difference to the standard deviation of percentages from their respective group mean) is 4.33, and the probability that as marked a difference as this could have been the result of chance is considerably less than 0.01. Thus, even with these small groups of values, a clear demonstration of an effect of muscular training in reducing the concentration of ether-soluble substances in the liver was obtained.

However, no other tissue showed a difference between exercised and unexercised steers sufficiently marked, in relation to the variation occurring within each group, to indicate that muscular training had produced it. The heart tissue, the special sample from the round, and the chuck muscle come the nearest to a demonstration, but the

probabilities that chance may have produced the average differences are so large (0.15, 0.13, and 0.13, respectively) that they can not be neglected. Even if all the muscle samples are considered together in two groups of 36 analyses each, and the deviations are computed from the averages of each group of four samples in arriving at the standard deviation, the probability obtained is greater than 0.10, and hence the possibility that chance was responsible for group differences can not be neglected.

In experiment 2, involving light work, the fat content of the livers of the exercised steers is again significantly lower than that of the unexercised steers, the average difference amounting to 0.91 per cent of fat. The value of t, according to Fisher's method, is 5.46, giving a probability for $n=6$ of considerably less than 0.01 that chance conditions alone brought about this distribution of values into the two groups. It may, therefore, be concluded that the work imposed upon the steers in one of these groups was responsible for lowering the content of ether-soluble constituents in the liver.

The heart samples also suggest strongly an effect of work similar to the effect upon the liver, but the probability in this case amounts to about 0.044 that chance factors may have brought this result about, equivalent to odds of 1 in 24. Any great amount of reliance can not, therefore, be placed on the significance of this comparison.

With respect to the fat contents of the muscle samples in experiment 2, nothing can be said concerning a possible effect of muscular training. Considering group averages only, in eight comparisons the exercised steers gave the lower figure, while in four comparisons the reverse was true.

Table 4 shows that with respect to all tissue samples taken in experiment 1, the water content on the protoplasmic basis averaged less for the exercised steers than for the unexercised steers. The difference was greatest and most distinct for the liver. amounting to 1.94 per cent of water. The value of t in this case is 3.90, and from Fisher's table (*11, p. 139*), only one value in a hundred (n being equal to 6) will exceed 3.707 by chance; hence, the difference is highly significant. It may, therefore, be concluded that continued exercise lowered the water content of the livers of these steers.

For a number of other samples, the differences between the two groups of steers in the percentage of water are significant, the value of P being less than 0.033, corresponding to odds of 1 in 30 that chance alone would produce the result, that is, heart 0.032, special sample of round 0.026, biceps femoris (round) muscle 0.032, and obliquus externus abdominis (flank) muscle 0.018. If the results on all the muscles are combined in a single statistical analysis, in which the standard deviation is computed from the deviations of each group of four percentages from its mean, the mean difference is 0.0668 per cent and the value of t becomes 7.67, n being 70. This value of t is far beyond the range of Fisher's table, so that it is perfectly clear that, as the statistician would say, the percentages of water in the muscles from the exercised and unexercised steers were not drawn from the same "population." Hence, it may be concluded that, continued muscular exercise of the intensity imposed in this experiment reduces the moisture content of liver, heart, and muscles.

In experiment 2 the percentages of water on the protoplasmic basis were also lower for the exercised steers with the exception of

two samples, the plate and the special sample of round, but the average differences are considerably smaller than those obtained in experiment 1 and, hence, less certainly caused by the difference in muscular activity. With only two samples is the probability that all analyses are from the same population sufficiently small that it may be neglected. For the liver samples, the average difference in water content was 2.22 per cent, the value of t being 4.24, and the probability considerably less than 0.01. Also for the rib samples the average difference was 1.13 per cent, the value of t 3.63, and the probability approximately 0.011. If all the muscle samples are combined in one analysis, as was done in the discussion of the corresponding results of experiment 1, the mean difference is 0.265 (as compared with 0.668), the value of t becomes 2.45 (as compared with 7.67), and P is somewhat less than 0.02. It appears, therefore, that in this experiment also the exercise imposed has markedly lowered the moisture content of liver and also that of the muscles, though to a less pronounced extent, than did the more severe muscular exercise imposed in experiment 1.

The analyses of fat and water in a number of tissues from 16 different steer calves offers an opportunity of studying statistically the relation between the composition of different muscular tissues in the same animal. This relationship is of general interest, and in addition it bears directly upon the interpretation of the data with respect to the effect of continued muscular exercise upon the fat and water content of the muscles, since it will show to what extent the analyses of the different samples are interdependent, and conversely, to what extent each sample analyzed is an independent test of the effect of muscular work. The product-moment correlation method was therefore applied to the water and fat analyses in each experiment, the standard deviations being computed from the deviations of each group of four analyses from its own mean. In computing the deviation porducts every possible pair of deviations was taken from all the muscle samples of each steer.

In experiment 1 the correlation coefficient for fat content was +0.360, and that for water content +0.182. In experiment 2 the correlation coefficients were, respectively, +0.519 and +0.346. These coefficients measure the degree to which variations in the fat and water content in one sample of muscle tissue are associated with variations in another sample, perfect correlation being measured by a coefficient of 1 and the positive sign indicating that a high content in one muscle tends to be associated with a high content in another. Evidently a closer correlation exists among the different tissues with respect to fat than with respect to water, and also, apparently, there is a closer correlation between the fat content, as well as the water content, of lean samples from the same carcass, freed roughly from visible fat with a knife, than between the fat content or the water content of individual muscles, dissected out in a manner better calculated to avoid contamination with intermuscular fat. The correlation coefficients obtained in the first experiment are inconsiderable, though significant in all probability in indicating the existence of some degree of positive correlation. The coefficients obtained in the second experiment are somewhat larger, though still too small to indicate that the fat or moisture content of one lean sample may be predicted satisfactorily from that of another.

BOUND WATER IN MUSCLE

In experiment 1 (heavy work) an attempt was made to determine whether trained muscle possessed a different percentage of "bound" water than untrained muscle. The conception of "bound" water followed was that of Rubner, according to which "bound" water is that fraction of the water in the tissue which does not freeze at temperatures as low as −20° C. The method of determining bound water was essentially that of Robinson (*23*), which is a modification of the method of Thoenes (*27*). The sample used for the determination was the special sample from the round, taken immediately after slaughter, and the determinations were made within 48 hours. The results are assembled in Table 5.

TABLE 5.—*Bound-water content of the special samples of round lean from steers on heavy work, experiment 1*

[All results are expressed on the fat-free basis]

Treatment and steer No.	Total water	Bound water			Treatment and steer No.	Total water	Bound water		
		As percentage of total water	As percentage of fat-free substance	Per gram of fat-free dry matter			As percentage of total water	As percentage of fat-free substance	Per gram of fat-free dry matter
Exercised:	*Per cent*	*Per cent*	*Per cent*	*Gram*	Unexercised:	*Per cent*	*Per cent*	*Per cent*	*Gram*
84	75.73	14.3	10.8	0.45	23	76.93	14.5	11.1	0.48
9	75.71	14.6	11.0	.45	21	77.23	11.9	9.2	.40
62	76.31	12.6	9.6	.41	43	77.25	12.1	9.3	.41
34	76.60	13.7	10.5	.45	36	76.44	13.2	10.1	.43
Average	76.09	13.8	10.5	.44	Average	76.96	12.9	9.9	.43

It is clear that no evidence was obtained of an effect of training on the bound-water content of muscle.

THE NITROGEN CONTENT OF THE FAT-FREE DRY MATTER OF THE TISSUES

The nitrogen content of the tissue samples in both experiments has been expressed on the basis of the fat-free dry matter, in order to eliminate the variations in fat and water, which have been shown to be, to some extent, influenced by muscular training. The percentages of nitrogen thus expressed are presented in Table 6.

In considering experiment 1 involving heavy work, first, it appears that for all tissues examined except two the average percentage of nitrogen for the exercised steers is lower than that for the unexercised. In one case, the semitendinosus muscle of the round, the averages for the two groups of steers are the same, while in another case, the biceps femoris of the round, there is a difference of 0.02 per cent in favor of the exercised steers.

As in all other cases in which training has affected the chemical composition of tissues, the composition of the liver is affected the most. In this instance, the average reduction of the nitrogen content of the fat-free dry matter is 1.33 per cent of nitrogen. Applying Fisher's method of statistical analysis, the standard deviation s is 0.502, t of 3.75, and the probability that the two sets of data are from the same population is less than 0.01. There can be little doubt that

muscular training has reduced the nitrogen content of the fat-free dry matter of the liver.

TABLE 6.—*Nitrogen content (per cent) of the fat-free dry matter of the tissues from steers on heavy and on light work, and from unexercised steers*

EXPERIMENT 1, HEAVY WORK

Treatment and steer No.	Heart	Liver	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
				Semimembranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triceps brachii
Exercised:											
84	14.68	11.12	14.91	14.70	14.90	14.86	14.46	16.01	14.90	15.00	14.50
9	14.67	11.59	15.17	14.72	15.48	15.65	15.25	15.15	15.08	15.34	14.82
62	14.98	11.26	15.20	15.19	15.69	15.04	14.88	15.36	15.27	14.81	15.07
34	15.42	11.18	15.24	15.03	15.63	15.32	14.84	15.62	15.24	15.26	14.99
Average	14.94	11.29	15.13	14.91	15.43	15.22	14.86	15.54	15.12	15.10	14.85
Unexercised:											
23	14.90	11.96	15.40	15.14	14.80	14.91	14.68	16.81	15.44	15.85	14.95
21	15.19	12.29	15.79	15.14	15.34	15.47	15.01	15.44	15.36	15.41	15.50
43	15.09	12.70	15.64	16.18	15.76	14.94	15.02	15.27	15.83	15.11	14.78
36	15.50	13.53	15.27	15.10	15.81	15.46	15.17	14.93	15.27	15.78	15.35
Average	15.17	12.62	15.53	15.39	15.43	15.20	14.97	15.61	15.48	15.54	15.15

EXPERIMENT 2, LIGHT WORK

Treatment and steer No.	Heart	Liver	Special sample of round	Round	Ribs	Fore shank	Loin end	Short loin	Rump	Plate	Chuck	Flank
Exercised:												
80	14.27	11.10	14.70	15.03	15.50	15.64	15.12	15.54	15.11	15.23	15.70	15.98
116	15.02	11.72	15.51	14.99	14.76	16.06	15.35	15.51	16.14	15.32	15.36	15.73
119	15.72	12.17	15.18	14.82	14.59	14.77	16.38	15.94	15.11	15.77	15.63	15.31
125	16.17	11.98	15.12	15.01	14.58	16.09	15.51	15.17	15.73	15.71	15.38	16.34
Average	15.30	11.74	15.13	14.96	14.86	15.64	15.59	15.54	15.52	15.51	15.52	15.84
Unexercised:												
103	.....	12.82	15.25	14.71	15.22	16.18	15.41	15.37	15.06	15.01	15.01	.....
117	14.52	13.18	14.41	15.35	15.60	15.72	15.90	15.81	15.88	15.33	15.53	15.29
121	14.99	13.30	15.60	14.81	15.00	15.32	15.14	16.24	15.20	15.27	15.14	15.71
129	14.36	13.17	14.89	14.29	15.26	15.64	15.23	15.38	15.56	15.63	15.28	15.03
Average	14.62	13.12	15.04	14.79	15.27	15.72	15.42	15.70	15.43	15.31	15.24	15.34

In the special sample of tissue from the round, there was an average difference of 0.40 per cent of nitrogen, a standard deviation of 0.196, a value of t is 2.88, and a probability of about 0.032 that the two sets of data are the result of the operation of the same factors. The indications are clear with this tissue also, muscular training has lowered the nitrogen content of the fat-free dry matter. The other muscle samples, secured from the chilled carcasses, do not individually show clear evidence of such an effect of training; the longissimus dorsi muscle of the ribs gives the most significant evidence. but the probability that the two groups of analyses may have been produced by

the same factors is too large (0.054) to be safely neglected. However, if all of these eight muscle samples are considered together in the same statistical analysis, computing the standard deviation from the deviations of each group of four analyses from its own mean, an average difference of 0.217 per cent is obtained between exercised and unexercised steers, the standard deviation is 0.334, t is 2.59, and the probability that the same factors operated in determining the percentage for both groups of steers is less than 0.02. Such a low probability of a chance outcome may be neglected, and it may be concluded that, in muscle tissue generally, the effect of muscular training is to lower the nitrogen content of the fat-free dry matter.

In experiment 2 (light work) the liver undoubtedly has suffered the same large reduction (1.38 per cent) in the nitrogen content of the fat-free dry matter. The value of t in this comparison is 5.40, and the probability is considerably less than 0.01. But the heart and the special round sample show no such relation, and of the nine muscle samples from the chilled carcasses only three show an average reduction in the percentage of nitrogen for the exercised steers. Evidently the intensity of the work imposed in this experiment was not sufficient to affect appreciably the nitrogen content of the fat-free dry matter.

THE GLYCOGEN CONTENT OF THE FRESH MUSCLE

In accounting for the reduction in the nitrogen content of the fat-free dry matter of the tissues brought about by muscular training and especially evident in the results of experiment 1, one naturally, perhaps, thinks first of an increase in glycogen. But it would require an improbably large increase in the glycogen content of the tissues to account for the decreases in nitrogen observed. In experiment 1, in which the average nitrogen content of the liver on the fat-free dry-matter basis decreased from 12.62 to 11.29 per cent, it would require an increase of glycogen equal to 10.54 per cent of the dry matter. In experiment 2, the average reduction in the nitrogen percentage in the liver was from 13.12 to 11.74, which would again call for an increase in glycogen equal to 10.52 per cent of the dry matter. In the case of the fresh round sample in experiment 1, the nitrogen content decreased on an average from 15.53 per cent of the fat-free dry matter to 15.13 per cent. If this had been brought about by an increased deposition of glycogen, the increase would have to equal 2.58 per cent of the fat-free dry matter.

Glycogen determinations were made upon the fresh samples of the round taken in experiment 1, using the usual method of alkali decomposition of the tissues and alcohol precipitation of the glycogen. The special technic recommended by Fisher and Lackey (*10*) was followed in these analyses. The results for exercised steers, in the order in which they have been arranged in the tables, are 3.92, 3.10, 3.63, and 3.73 per cent of glycogen on the fat-free dry matter basis, averaging 3.60 per cent. For the unexercised steers the results are, in the order given in the tables, 2.96, 1.48, 2.45, and 3.28 per cent of glycogen, averaging 2.54 per cent. These values indicate that muscular training may have increased the glycogen content of the dry muscle. Statistical analysis gives a probability of 0.05 that the two series of analyses resulted from the same combination of factors, so that the above interpretation can not be said to have been demonstrated.

THE LIPOID CONSTITUENTS OF THE TISSUES

Since an accumulation of glycogen in the tissues of the trained animals can not reasonably account in full for the reduction in the nitrogen content of the fat-free dry matter, another possibility is that there is an increased content of lipoids in the tissues of the trained animals. It is known that ether will not extract all the lipoids from tissues. Although none of the data obtained in this investigaiton can throw any direct light upon this question, reference may be made, in support of the explanation offered above, to the work of Bloor (*3*) ,who, in investigations of the lipoid content of a few selected beef muscles, found that the contents of cephalin and lecithin varied from muscle to muscle in the order of what might be considered to be their activity, the more active the muscle the higher its percentage content of phospholipid. Furthermore, Cowdry (*6*) in a recent discussion of the mitochondrial constituents of protoplasm (in all probability largely phospholipid in character), has noted the association of mitochondria with intense protoplasmic activity. He says (*6, p. 82*):

> In cytomorphosis, for example, they are especially numerous in the active stages in the life of the cell and they diminish with senility in both plants and animals. There is a sharp increase in mitochondria with regenerative activity, in compensatory hypertrophy, and in many other conditions.

Of particular interest to the interpretation of the results of these experiments are the following observations of Cowdry:

> * * * there is a distinct reciprocal relationship between the amount of mitochondria and the amount of fat. Where there are few mitochondria there is much fat, and *vice versa*. Decreased oxidation favors the accumulation of fat and increased oxidation favors its elimination, which suggests at once some connection between the amount of mitochondria and oxidation; and their abundance in the active stages of the life of the cell, where protoplasmic respiration is rapid, points to the same conclusion.

Hence, the decrease in ether-soluble constituents observed in the exercised as compared with the unexercised steers (Table 4) may be considered indirect evidence of an increased phospholipid content.

CREATINE CONTENT OF THE TISSUES

In experiment 1 creatine was determined in all the tissues. In experiment 2 only six of the samples were thus analyzed. The results obtained have been summarized in Table 7. They are expressed (1), on the protoplasmic basis to eliminate the effect of variations in the fat content of the tissues, (2) on the fat-free and water-free basis, to eliminate the effect of variations in both the fat and the water content of the tissues, and (3) for the data of experiment 1 only the creatine nitrogen is expressed as a percentage of the total nitrogen.

TABLE 7.—*Creatine content of the tissue samples from steers on heavy and on light work, and from unexercised steers*

EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF CREATINE ON THE PROTOPLASMIC BASIS

Treatment and steer No.	Heart	Liver	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
				Semimembranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triceps brachii
Exercised:											
84	0.274	0.010	0.440	0.461	0.567	0.466	0.460	0.619	0.469	0.456	0.406
9	.232	.020	.380	.402	.546	.484	.420	.627	.424	.399	.427
62	.234	.020	.380	.431	.563	.461	.441	.564	.448	.405	.402
34	.238	.024	.389	.499	.518	.465	.467	.554	.485	.449	.419
Average	.245	.019	.397	.448	.549	.469	.447	.591	.457	.427	.414
Unexercised:											
23	.245	.012	.515	.434	.525	.443	.414	.519	.450	.463	.381
21	.263	.020	.470	.437	.577	.473	.477	.663	.477	.473	.439
43	.263	.044	.428	.461	.586	.469	.437	.533	.426	.416	.444
36	.276	.058	.439	.511	.639	.503	.484	.597	.491	.434	.448
Average	.262	.034	.463	.461	.582	.472	.453	.578	.461	.447	.428

EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF CREATINE ON THE FAT-FREE AND WATER-FREE BASIS

Exercised:											
84	1.41	0.035	1.81	1.93	2.37	1.97	1.90	2.76	2.03	1.97	1.73
9	1.17	.071	1.56	1.65	2.29	2.03	1.77	2.72	1.79	1.65	1.82
62	1.24	.071	1.60	1.81	2.38	1.96	1.83	2.56	1.94	1.72	1.77
34	1.26	.084	1.66	2.09	2.16	1.98	1.96	2.50	2.09	1.88	1.87
Average	1.27	.065	1.66	1.87	2.30	1.99	1.87	2.64	1.96	1.81	1.80
Unexercised:											
23	1.33	.044	2.23	1.84	2.20	1.91	1.76	2.40	1.96	2.10	1.76
21	1.43	.074	2.06	1.87	2.51	2.08	2.02	3.06	2.07	2.12	1.97
43	1.48	.166	1.88	2.07	2.54	2.00	1.87	2.44	1.95	1.78	1.97
36	1.46	.224	1.86	2.15	2.70	2.22	2.02	2.70	2.09	1.91	1.96
Average	1.42	.127	2.01	1.98	2.49	2.05	1.92	2.65	2.02	1.98	1.92

EXPERIMENT 1, HEAVY WORK, CREATINE NITROGEN AS A PERCENTAGE OF THE TOTAL NITROGEN

Exercised:											
84	3.16	0.101	3.94	4.25	5.16	4.32	4.27	5.67	4.45	4.36	3.87
9	2.64	.197	3.37	3.68	4.85	4.23	3.84	6.02	3.93	3.63	4.03
62	2.73	.201	3.42	3.85	4.93	4.27	3.98	5.46	4.14	3.81	3.85
34	2.70	.240	3.54	4.52	4.51	4.21	4.34	5.30	4.49	4.05	4.07
Average	2.81	.185	3.57	4.08	4.86	4.26	4.11	5.61	4.25	3.96	3.96
Unexercised:											
23	2.96	.119	4.72	3.96	4.84	4.20	3.93	4.80	4.15	4.37	3.92
21	3.12	.193	4.26	4.03	5.35	4.37	4.39	6.56	4.42	4.57	4.16
43	3.22	.430	3.92	4.21	5.25	4.37	4.06	5.26	4.05	3.88	4.37
36	3.14	.580	3.98	4.64	5.58	4.70	4.36	5.98	4.48	4.00	4.21
Average	3.11	.323	4.22	4.21	5.26	4.41	4.19	5.65	4.28	4.21	4.17

EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF CREATINE ON THE PROTOPLASMIC BASIS

Treatment and steer No.	Liver	Heart	Special sample of round	Round	Ribs	Shank
Exercised:						
80	0.041	0.261	0.441	0.411	0.418	0.358
116	.041	.228	.434	.392	.287	.291
119	.043	.233	.459	.407	.401	.346
125	.047	.226	.425	.432	.394	.332
Average	.043	.237	.440	.411	.375	.332
Unexercised:						
103	.032	.223	.471	.423	.411	.347
117	.035	.265	.476	.384	.393	.322
121	.053	.288	.487	.413	.406	.371
129	.041	.295	.471	.416	.432	.364
Average	.040	.268	.476	.409	.411	.351

EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF CREATINE ON THE FAT-FREE AND WATER-FREE BASIS

Exercised:						
80	0.139	1.30	1.79	1.70	1.76	1.46
116	.142	1.22	1.80	1.67	1.19	1.28
119	.152	1.22	1.90	1.72	1.64	1.43
125	.172	1.22	1.77	1.80	1.60	1.41
Average	.151	1.24	1.82	1.72	1.55	1.40
Unexercised:						
103	.121	1.23	1.98	1.77	1.81	1.52
117	.132	1.30	1.93	1.67	1.73	1.38
121	.206	1.52	2.04	1.74	1.74	1.59
129	.153	1.57	1.89	1.71	1.82	1.51
Average	.153	1.43	1.96	1.72	1.78	1.50

Of the 11 tissue samples analyzed for creatine in experiment 1, 10 showed smaller average percentages of creatine on the protoplasmic basis for the exercised steers than for the unexercised. While this outcome is suggestive of an effect of muscular training it falls short of a demonstration. The average differences are in the main slight, and the application of statistical analysis, either to the analytical results of individual tissues or of all muscular tissues combined, does not give any clear indication that the creatine content of the tissues of the two groups of steer calves was determined by any factors but those common to both groups. It is worthy of note that the creatine percentages for the different muscle samples are all of about the same order of magnitude, except those for the semitendinosus of the round and the psoas major of the short loin, which contain more than 0.1 per cent more creatine.

The percentages of creatine expressed on the fat-free and water-free basis for experiment 1 offer clearer evidence of a depressing effect of muscular training on the creatine content. All tissue samples show a lower average percentage of creatine for the exercised steers. Statistical analysis of the results of individual tissues reveals a decisive result only in the case of the special sample of round. Here the average difference is 0.35, the standard deviation is 0.145, t is 3.41, and the probability is about 0.015 that the values for the two groups of steers were the result of common factors only. It seems fair to neglect this small probability and to conclude that with respect to this sample some factor operating in one group but not in the other

accounted for the average depression in creatine content. This factor could only have been the muscular work imposed on one of the groups of steers.

A comparison of the creatine percentages of the two groups of steers with respect to heart muscle indicates a probability of slightly less than 0.05 that a random operation of common factors alone determined the difference between groups. While this probability is too large to be neglected, it is highly suggestive that muscular training was involved in the production of the lower percentages for the exercised steers.

A further statistical analysis was made by combining all the analyses of muscle samples that contain approximately the same percentage of creatine, except the special sample from the round which gave significant results by itself. The two muscles with a high creatine content, the semitendinosus and the psoas major, were thus excluded. For the remaining six muscles, the unexercised steers gave an average creatine content of 1.9771 per cent of the fat-free dry matter, while the exercised steers gave an average of 1.8808 per cent, a difference of 0.0963. The standard deviation from these two averages is 0.1260, and the value of t is 2.644; for this value of t and n is 46, the probability desired is about 0.01. It may, therefore, be concluded that the preponderance of evidence shows that prolonged muscular training has lowered the creatine content of the muscular tissues generally.

The creatine calculations expressed as percentages of creatine nitrogen on the total nitrogen do not reveal any more distinct differences than do the percentages of creatine in the fat-free dry matter.

It is noteworthy that the liver does not show any significant differences in creatine content on any basis of calculation, in contrast with all other comparisons, in which the effect of muscular training was more marked and clear cut with respect to the liver than with respect to any other tissue. This is true not only for the data of experiment 1, but also for the data of experiment 2.

In experiment 2, as in the first experiment, the special sample of round muscles shows the most marked effect of muscular training. The percentages of creatine on the protoplasmic basis show an average difference of 0.036, a standard deviation of 0.0115, and a value of t of 4.43, indicating a probability of considerably less than 0.01 that a chance combination of factors common to both groups of steers accounts for the average difference observed. This probability is small enough to be neglected. For the same sample, the average percentages of creatine on the fat-free and water-free basis for the two groups differ by 0.14 , the standard deviation is 0.0616, the value of t is 3.21, and the probability is about 0.019 that chance factors only were responsible for the group difference. Again, this is small enough to be neglected. The probability that chance factors accounted for the difference between average percentages of creatine for the heart is about 0.05.

If a single analysis is made of the creatine results for the round, ribs, and shank, the probability that the average percentages on the protoplasmic basic are the result of common factors only is about 0.10, while, for the average percentages on the fat-free dry matter, the probability is 0.02 ($t=2.52$, $n=22$). The latter probability is sufficiently low to neglect. It may be concluded, therefore, that in

this as well as in the other experiment, the data indicate clearly that prolonged muscular training depresses the creatine content of muscle, an effect that is most clearly discernible only when variations in the fat and water content of the muscle have been eliminated by computing the creatine content as a percentage of the fat-free dry matter.

In order to determine the extent to which creatine percentages on the protoplasmic basis in one muscle sample were related to creatine percentages in other muscle samples from the same carcass, correlations were made between deviations of each group of four percentages from its own mean, and deviations of all other groups, making all possible pairings of deviations relating to the same steer. In experiment 1 288 such pairings were made from all the muscle samples except the heart, and the correlation coefficient is +0.206, perfect correlation being represented by +1. For the four muscle samples of experiment 2, there were 48 possible pairings, and the coefficient of correlation is +0.406. Putting its rightful emphasis upon the former coefficient, it may be concluded that only a slight correlation exists between the creatine content of one muscle and that of another in the same carcass.

COLLAGEN AND ELASTIN CONTENT OF THE MUSCLES

The results of the determinations of collagen and elastin in the muscle tissue, expressed as collagen and elastin nitrogen in per cent of the total nitrogen, are summarized and averaged in Table 8.

TABLE 8.—*Collagen and elastin contents of the tissue samples from steers on heavy and on light work and from unexercised steers*

EXPERIMENT 1, HEAVY WORK, COLLAGEN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Treatment and steer No.	Heart	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
			Semimembranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triceps brachii
Exercised:										
84	11.6	7.5	5.5	5.2	4.6	3.1	4.6	2.9	6.7	5.5
9	9.3	13.6	6.3	7.7	7.0	5.9	6.8	4.4	10.2	7.4
62	9.5	4.0	6.3	11.8	8.4	5.1	5.3	4.4	8.4	6.6
34	13.3	7.8	5.3	14.0	6.9	4.8	10.4	4.6	9.2	10.3
Average	10.9	8.2	5.8	9.7	6.7	4.7	6.8	4.1	8.6	7.4
Unexercised:										
23	12.8	8.5	5.4	5.2	4.8	3.2	4.2	3.1	6.0	6.3
21	11.8	7.5	6.4	8.1	9.1	6.8	6.6	5.6	7.3	8.3
43	12.8	4.1	7.1	10.7	9.3	5.8	7.6	5.3	8.9	8.3
36	11.9	8.5	5.0	11.4	7.8	4.4	9.1	5.6	8.6	10.1
Average	12.3	7.2	6.0	8.8	7.8	5.0	6.9	4.9	7.7	8.2

EXPERIMENT 1, HEAVY WORK, ELASTIN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Exercised:										
84	0.26	.....	0.070	1.7	0.23	0.062	0.102	0.056	0.143	0.095
9	.32	0.16	.112	2.5	.10	.042	.061	.046	.100	.042
62	.30	.11	.082	2.0	.16	.051	.057	.095	.087	.110
34	.11	.26	.116	3.3	.10	.080	.108	.049	.200	.079
Average	.25	.18	.095	2.4	.15	.059	.087	.062	.132	.082
Unexercised:										
23	.36	.12	.112	2.8	.20	.077	.089	.055	.135	.106
21	.38	.14	.098	3.0	.14	.064	.063	.075	.090	.047
43	.35	.13	.082	2.9	.16	.054	.049	.056	.112	.109
36	.32	.09	.084	2.4	.13	.074	.058	.044	.136	.072
Average	.35	.12	.094	2.8	.16	.067	.065	.058	.118	.081

TABLE 8.—*Collagen and elastin contents of the tissue samples from steers on heavy and on light work and from unexercised steers*—Continued

EXPERIMENT 2, LIGHT WORK, COLLAGEN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Treatment and steer No.	Heart	Special sample of round	Round	Ribs	Shank	Loin end	Short loin	Rump	Plate	Chuck	Flank
Exercised:											
80	7.5	5.5	12.4	11.2	.....	8.6	11.5	11.2	15.4	15.7	13.6
116	8.8	5.2	9.5	12.1	.....	9.7	9.3	9.5	14.3	14.3	14.1
119	7.8	5.3	12.8	13.7	24.7	8.4	11.6	11.1	15.6	15.5	12.4
125	8.3	4.2	12.0	7.8	23.1	12.0	11.2	12.4	14.4	16.1	12.6
Average	8.1	5.0	11.7	11.2	23.9	9.7	10.9	11.0	14.9	15.4	13.2
Unexercised:											
103	.....	8.4	10.7	13.8	23.3	11.4	12.2	13.2	12.8	14.8	.....
117	11.8	5.1	12.6	13.4	26.4	11.6	11.2	11.0	16.0	15.7	14.9
121	8.0	7.9	11.1	8.3	21.1	10.5	11.8	11.6	14.4	16.3	16.4
129	7.8	5.3	12.0	13.1	21.6	11.7	10.2	13.4	14.0	16.3	10.6
Average	9.2	6.7	11.6	12.2	23.1	11.3	11.4	12.3	14.3	15.8	14.0

EXPERIMENT 2, LIGHT WORK, ELASTIN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Exercised:											
80	0.20	0.082	0.48	1.03	.....	0.16	0.43	0.26	2.0	0.49	1.71
116	.22	.110	.37	.81	0.08	.12	.22	.18	1.2	.40	.69
119	.20	.116	.42	.92	.25	.18	.40	.20	1.6	.40	1.33
125	.20	.062	.47	.94	.36	.11	.38	.20	1.4	.49	1.08
Average	.20	.092	.44	.92	.23	.14	.36	.21	1.6	.44	1.20
Unexercised:											
103	.....	.118	.58	.99	.10	.20	.43	.28	2.1	.57	.....
117	.36	.180	.29	.93	.15	.17	.46	.21	1.6	.49	1.34
121	.39	.101	.25	.70	.20	.14	.29	.21	1.2	.43	.96
129	.19	.097	.71	1.10	.32	.10	.36	.30	2.0	.51	1.09
Average	.31	.124	.46	.93	.19	.15	.38	.25	1.7	.50	1.13

It was evident during the making of these determinations that the collagen results were too high, due to the inclusion in the gelatin filtrates of nongelatin material identified by its content of tryptophane. The determinations of experiment 2 were run in no orderly fashion, but those of experiment 1, undertaken the following year, were run in pairs, so that, for each muscle tissue, a sample from an exercised and an unexercised steer were run simultaneously. In each determination, the gelatin filtrate was tested for tryptophane by the Ehrlich colorimetric reagent, and each test was graded roughly according to the intensity of color as faint, medium, and strong. When these tests were assembled and compared at the end of the experiment, it was evident that there was a strong tendency for determinations made simultaneously to give similar tryptophane colorations in the gelatin filtrates. Of 39 such simultaneous determinations on which color gradings were recorded, 37 were given equal gradings and only 2 unequal gradings. It thus appears that, although all collagen determinations are too high, those run simultaneously, having been submitted to more nearly equal conditions of time and temperature, contain similar contaminations of nongelatin nitrogen in the gelatin filtrate and hence are comparable.

The steers in experiment 1 were paired for collagen determinations as follows: Nos. 84 and 23, 9 and 21, 62 and 43, and 34 and 36.

When the determinations are thus paired, and the result for the exercised steer of each pair is subtracted from the result for the unexercised steer, the differences collected in Table 9 are obtained.

TABLE 9.—*Differences in the percentage of collagen nitrogen between the tissues from pairs of steers upon which determinations were made simultaneously, Experiment 1, heavy work*

Pair composed of steers, Nos.	Heart	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
			Semimembranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triceps brachii
84 and 23	1.17	1.05	−0.14	0.04	0.18	0.09	−0.34	0.21	−0.70	0.73
9 and 21	2.52	−6.10	.11	.41	2.06	.96	−.25	1.12	−2.82	.87
62 and 43	3.32	.13	.80	−1.09	.86	.72	2.29	.94	.56	1.70
34 and 36	−1.46	.72	−.28	−2.53	.89	−.40	−1.30	1.02	−.62	−.22

It will be noted that, of the 40 differences, 26 are positive and 14 are negative, indicating that in general the muscles from the exercised steers contained less collagen than the muscles from the unexercised steers. Furthermore, one of the negative differences involves an aberrant result on the special round sample of one of the exercised steers (No. 9), a result that is much higher than any other and may represent a different type of muscle sample than the others, including a different selection of muscles or possibly the inadvertent inclusion of more tendinous tissue.

If all of the differences, with the exception of the difference above noted representing the special round comparison of steers 9 and 21, are averaged, a result of $+0.29 \pm 0.13$ is obtained. Since this difference is only a little more than twice its probable error, it is not statistically significant, but it is nevertheless suggestive of an effect of muscular training.

Among the comparisons of collagen analyses on the individual muscles, only two, that for the biceps femoris of the round and the longissimus dorsi of the ribs, indicate a significant difference between exercised and unexercised steers or a difference approaching significance. In the case of the biceps femoris, the average difference is $+1.00$, the standard deviation 0.676, the ratio of the two, z, is 1.5, and the probability according to Student is only 0.04 that the average is a result of chance. For the longissimus dorsi muscle of the ribs, the average difference is $+0.82$, the standard deviation 0.36, the ratio z is 2.3, and the probability is only 0.014. If the differences representing the longissimus dorsi muscle in the rib cut and in the short loin are combined, the mean difference is $+0.71$, the standard deviation 0.53, z is 1.33, and the probability that the mean is the result of a random combination of factors common to both groups of steers is only 0.0049. This and the preceding probability are so small as to be negligible, so that it may be concluded that for this muscle at least the evidence is clear that muscular training has decreased the collagen content. In the case of no muscle was there any indication approaching significance that muscular training had increased the percentage of collagen nitrogen.

The elastin analyses of experiment 1 do not indicate any general significant effect of muscular training, except with respect to the heart muscle. Here, in every pair of analyses, the result on the unexercised steer was greater than that on the exercised, the average difference being 0.10 per cent. The standard deviation of the four differences is 0.0634, the ratio z is 1.66, and the probability that chance produced the average difference is only 0.033.

In experiment 2, involving the lighter intensity of work there are no clear indications of an effect of muscular training, although again there are suggestions that the percentage of collagen nitrogen, and also of elastin nitrogen, have been depressed by work. In 8 of the 11 samples of muscle analyzed, the average percentage of collagen nitrogen was lower for the exercised steers than for the unexercised, and in 9 of the 11 samples the average percentage of elastin nitrogen was lower for the exercised than for the unexercised steers. However, the variations among individual analyses are too great to permit any positive interpretation of these average differences.

An interesting confirmation of these results on the collagen contents of the muscles of exercised and unexercised steers are the results obtained on the tenderness of the cooked ribs obtained from the half carcasses not submitted to chemical study. This work was done by the United States Department of Agriculture. A committee trained in the grading of the various factors contributing to the palatability of cooked meat, including its tenderness, tested all of the 16 rib roasts[6] from the steers of this experiment. In addition, the cooked roasts were subjected to a mechanical test for tenderness employing the method described in Technical Bulletin 217 of the United States Department of Agriculture (*2*). Through the courtesy of the department the writers have been permitted to refer to these results here in so far as they relate to tenderness. In a series of roasts cooked by a carefully standardized method as these were, it is to be expected that tenderness would vary inversely with the collagen content of the raw meat.

In 1929, when the experiment involved the lesser degree of exercise, there was an indication that the meat from the exercised cattle was more tender than that from the unexercised cattle. This conclusion is based upon both the average committee gradings and the average resistances of the meat to the mechanical device. However, variation among individual roasts, together with the small number involved in each group, detracts from the significance of this indication.

In the 1930 experiment, when there was much more exercise involved and the eight animals were paired, there was a greater difference in tenderness between the exercised and unexercised cattle as shown by the average committee gradings and mechanical tests on the cooked roasts. With three of the four pairs the judges found the meat from the exercised cattle more tender than that from the unexercised cattle. With the other pair the difference was too small to be regarded as of any significance. For that pair of roasts the mechanical test showed, in fact, that exercise had made the meat less tender. With this exception the mechanical test supported the gradings by the judges.

In addition to the roast, a round steak was cooked from each of the 16 cattle in the two years' work. In 1929 the steaks were from the

[6] These rib roasts were taken from the half carcasses not submitted to chemical analysis.

heel of the round and were somewhat lacking in uniformity, due to variations in cutting. For this and other reasons it was impossible to cook (broil) the steaks by a method as well standardized as that employed in roasting the rib cuts. Therefore the value of the data may be questioned but they suggest results in accord with those obtained from the rib cuts from the same experiment.

The round steaks in 1930 were uniformly cut and were taken from a more desirable part of the round than were those of 1929. Again the method of broiling was not standardized, but it is believed that there was greater uniformity in the cooking of the eight steaks than in 1929. Considering both committee gradings and mechanical tests of tenderness the data suggest that the steaks from the exercised cattle were slightly more tender than those from the unexercised cattle.

Any general statement of results must take into account the small total number of animals involved, the variability observed, and the lack of precision in the methods available for measuring tenderness. Making allowance for them, the data indicate that exercise was a factor contributing to tenderness, but the results can not be looked upon as altogether conclusive. When exercise was light, the comparative figures suggested that exercise increased tenderness; when exercise was heavy, the comparative figures showed a slightly greater association of tenderness with exercise.

ASH CONTENT OF THE TISSUES

The tissue samples obtained in experiment 1 were analyzed for total ash, but since the results were very uniform and disclosed no effect of muscular training they will not be given in detail. Expressed on the protoplasmic basis, the ash content of the heart samples averaged 1.075 per cent, that of the livers, 1.435 per cent, and that of the 72 muscle samples 1.145 per cent. The coefficient of variation of the latter group of results was only 4.45.

SUMMARY AND CONCLUSIONS

The effect of muscular activity on the chemical composition of the tissues of Hereford steer calves has been investigated in experiments covering 2 years. Each experiment involved 8 calves, 4 closely confined and 4 exercised daily on a treadmill. The exercise was continued for 122 days in one year and for 131 days in the other. In one experiment the muscular work imposed was much more intense than in the other, the working period averaging about 3 hours daily as compared with 1 hour daily. All steers were slaughtered at weights of 750 to 850 pounds, and samples of blood, heart, liver, and muscles were taken for analysis. In one experiment the total lean from each of the nine wholesale cuts from one-half of the carcass, separated from all visible fat by knife, constituted the muscle samples. In the other experiment, eight selected muscles were analyzed separately, and in both experiments a special sample of the inner and upper round were taken from the fresh carcasses for immediate study. The results obtained warrant the following statements with reference to the effect of long-continued muscular activity on the chemical characteristics of beef muscle and of other tissues:

No effect on the water, iron, hemoglobin, or red-cell content of the blood was produced.

No effect on the iron and hemoglobin content of the tissues was noted except the iron content of the bone marrow. In the one experiment (light work) in which this tissue was examined, muscular work clearly and markedly reduced the iron content.

While the results of the fat determinations suggested that muscular work tends to lower the content of ether-soluble material in all tissues, only the liver showed a clear and decisive effect, evident in both experiments. The results for the heart muscle were more nearly decisive than those for any other muscle sample.

In the experiment involving the heavier degree of work the evidence is clear that work lowers the content of water in liver, heart, and skeletal muscle, the effect on the liver being the most pronounced. The lighter work markedly lowered the water content of the liver, but its effect on the water contained in the muscles was less evident than in the other experiment. These findings were revealed by calculating the water content to the fat-free, so-called protoplasmic, basis.

No evidence was obtained that the condition of the water in muscle, that is, whether bound or free as measured by the heat-of-fusion method, was at all affected by muscular work.

The heavier degree of work distinctly lowered the nitrogen content of the fat-free dry substance in the liver and exerted a general though less distinct depression of this percentage in the muscles. The effect of the lighter degree of work was only evident in the liver. It appears probable that this general reduction in the nitrogen content of the dry substance of the tissues produced by the heavier degree of work is the result of three changes: (1) A decrease in nitrogenous extractives; (2) an increase in glycogen; and (3) an increase in lipoids not extractable by ether.

In both experiments the evidence indicates clearly that prolonged muscular training depresses the creatine content of muscle, an effect that is most clearly discernible only when variations in the fat and water content have been eliminated by computing the creatine content as a percentage of the fat-free dry substance. The muscle sample taken from the carcass immediately after slaughter and analyzed immediately showed this effect of work more clearly than did the muscle samples taken from the chilled carcasses.

Muscular work tends to lower the collagen content of muscle, expressed as a percentage of collagen nitrogen on the total nitrogen. Possibly the muscular work induced a hypertrophy of muscle tissue with no corresponding increase in the connective tissue elements.

The ash content of the tissues was not appreciably affected by the kind and degree of muscular work imposed in these experiments.

LITERATURE CITED

(1) Awdejewa, M. S., Gerasimovitch, W. W., Iwanowa, E. J., Messinewa, N. A., Prowatorowa, E. L., and Sawtisch, N. G.
1930. Die chemischen und physikalish-chemischen eigenschaften des blutes beim rinde, ihre schwankungen und individuellen unterschiede. Ztschr. Tierzüchtung u. Züchtungsbiol. 19 : 128–149, illus.

(2) Black W. H., Warner, K. F., and Wilson, C. V.
1931. Beef production and quality as affected by grade of steer and feeding grain supplement on grass. U. S. Dept Agr. Tech. Bul. 217, 44 p., illus.

(3) Bloor, W. R.
1927. DISTRIBUTION OF UNSATURATED FATTY ACIDS IN TISSUES. II. VOLUNTARY MUSCLE OF BEEF. Jour. Biol. Chem. 72 : 327–343.
(4) Bonnier, G., Jorpes, E., and Sköld, E.
1929. A STUDY OF SOME BLOOD COMPONENTS IN CATTLE. Ztschr. Tierzüchtung u. Züchtungsbiol. 13 : [343]–370.
(5) Brown, T. G., and Cathcart, E. P.
1909. THE EFFECT OF WORK ON THE CREATINE CONTENT OF MUSCLE. Biochem. Jour. 4 : 420–426.
(6) Cowdry, E. V.
1918. THE MITOCHONDRIAL CONSTITUENTS OF PROTOPLASM. Carnegie Inst. Wash. Contrib. Embryology 8 (25) : 39–160, illus.
(7) Drastich, L.
1928. LE TAUX DE L'HÉMOGLOBINE DANS LES HÉMATIES EST-IL CONSTANT CHEZ TOUS LES ANIMAUX? Compt. Rend. Soc. Biol. [Paris] 98 : 266–267.
(8) Embden, G., and Habs, H.
1927. ÜBER CHEMISCHE UND BIOLOGISCHE VERÄNDERUNGEN DER MUSKULATUR NACH ÖFTERS WIEDERHOLTER FARADISCHER REIZUNG. I. MITTEILUNG. Hoppe-Seyler's Ztschr. Physiol. Chem. 171 : [16]–39.
(9) Ferdmann, D., and Feinschmidt, O.
1929. ÜBER DEN EINFLUSS DES TRAINIERENS DES MUSKELS AUF SEINEN GEHALT AM PHOSPHORVERBINDUNGEN. Hoppe-Seyler's Ztschr. Physiol. Chem. 183 : 261–268.
(10) Fisher, N. F., and Lackey, R. W.
1925. THE GLYCOGEN CONTENT OF THE HEART, LIVER AND MUSCLE OF NORMAL AND DIABETIC DOGS. Amer. Jour. Physiol. 72 : 43–49.
(11) Fisher, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 p., illus. Edinburgh and London.
(12) Gerhartz, H.
1910. UNTERSUCHUNGEN ÜBER DEN EINFLUSS DER MUSKELARBEIT AUF DIE ORGANE DES TIERISCHEN ORGANISMUS, INSBESONDERE IHREN WASSERGEHALT. Pflüger's Arch. Physiol. 133 : [397]–499, illus.
(13) Hayden, C. E., and Fish, P. A.
1928. THE NORMAL BLOOD OF SOME DOMESTICATED ANIMALS. Cornell Vet. 18 : 197–203.
(14) Kennedy, R. P.
1927. THE QUANTITATIVE DETERMINATION OF IRON IN TISSUES. Jour. Biol. Chem. 74 : 385–391, illus.
(15) Kuhl, P.
1919. DAS BLUT DER HAUSTIERE MIT NEUEREN METHODEN UNTERSUCHT. I. UNTERSUCHUNG DES PFERDE-, RINDER- UND HUNDEBLUTES. Pflüger's Arch. Physiol. 176 : [263]–284, illus.
(16) McCay, C. M.
1931. THE HEMOGLOBIN AND TOTAL PHOSPHORUS IN THE BLOOD OF COWS AND BULLS. Jour. Dairy Sci. 14 : 373–378.
(17) Marloff, R.
1919. DIE FRÜHEREN ZÄHLUNGEN DER ERYTHROCYTEN IM BLUTE VERSCHIEDENER TIERE SIND TEILWEISE MIT GROSSEN FEHLEREN BEHAFTET. Pflüger's Arch. Physiol. 175 : [355]–370.
(18) Mellanby, E.
1908. CREATIN AND CREATININ. Jour. Physiol. 36 : [447]–487, illus.
(19) Mitchell, H. H., Hamilton, T. S., and Haines, W. T.
1928. SOME FACTORS AFFECTING THE CONNECTIVE TISSUE CONTENT OF BEEF MUSCLE. Jour. Nutrition 1 : 165–178.
(20) Newcomer, H. S.
1919. ABSORPTION SPECTRA OF ACID HEMATIN, OXYHEMOGLOBIN, AND CARBON MONOXIDE HEMOGLOBIN. A NEW HEMOGLOBINOMETER. Jour. Biol. Chem. 37 : 465–496, illus.
(21) Palladin, A., and Ferdmann, D.
1928. ÜBER DEN EINFLUSS DES TRAININGS DER MUSKELN AUF IHREN KREATINGEHALT. Hoppe-Seyler's Ztschr. Physiol. Chem. 174 : [284]–294.

(22) PROCTER, H. A., and BEST, C. H.
1932. CHANGES IN MUSCLE GLYCOGEN ACCOMPANYING PHYSICAL TRAINING. Amer. Jour. Physiol. 100 : 506–510.
(23) ROBINSON, W.
1928. RELATION OF HYDROPHILIC COLLOIDS TO WINTER HARDNESS OF INSECTS. *In* Weiser, H., ed., Colloid Symposium Monograph v. 5, p. 199–218, illus. New York.
(24) ROGOZINSKI, F.
1906. ÜBER DEN EINFLUSS DER MUSKELARBEIT AUF GEWICHT, ZUSAMMENSETZUNG UND WASSERGEHALT DER ORGANE DES TIERKÖRPERS. Biochem. Ztschr. 1 : [207]–228.
(25) ROSE, W. C., HELMER, O. M., and CHANUTIN, A.
1927. A MODIFIED METHOD FOR THE ESTIMATION OF TOTAL CREATININE IN SMALL AMOUNTS OF TISSUES. Jour. Biol. Chem. 75 : 543–548.
(26) SCHENKENBACH, H.
1926. DER EINFLUSS DES WEIDEGANGS AUF DIE BILDUNG VON ROTEN BLUTKÖRPERCHEN IM VERGLEICH ZUR STALLHALTUNG. Breslau. (Inaug.-Diss.)
(27) THOENES, F.
1925. UNTERSUCHUNGEN ZUR FRAGE DER WASSERBINDUNG IN KOLLOIDEN UND TIERISCHEN GEWEBEN. Biochem. Ztschr. 157 : [174]–186.
(28) VAN SLYKE, D. D., and STADIE, W. C.
1921. THE DETERMINATION OF THE GASES OF THE BLOOD. Jour. Biol. Chem. 49 : 1–42.
(29) WAGENAAR, M.
1930. ÜBER DEN NACHWEIS DER BLUTMENGE. Ztschr. Analyt. Chem. 81 : 207–212.
(30) WELLS, J. J., and SUTTON, J. E.
1915. BLOOD COUNTS IN THE FROG, THE TURTLE AND TWELVE DIFFERENT SPECIES OF MAMMALS. Amer. Jour. Physiol. 39 : 31–36.
(31) WHIPPLE, G. H.
1926. THE HEMOGLOBIN OF STRIATED MUSCLE. I. VARIATIONS DUE TO AGE AND EXERCISE. Amer. Jour. Physiol. 76: 693–707.

THE DEVELOPMENT AND FUNCTIONAL ACTIVITIES OF THE ALBINO RAT AS AFFECTED BY DIETS DEFICIENT IN IRON [1]

By THOMAS B. KEITH, *Instructor in Animal Husbandry*, and RUSSELL C. MILLER, *Associate in Animal Nutrition, Institute of Animal Nutrition, Pennsylvania State College* [2]

INTRODUCTION

This study seeks to throw light on the general subject of the functions of iron in nutrition. The experimental animal was albino rat, and the iron was supplied in natural foodstuffs. An iron-poor diet, the source of the protein and the iron of which is dried skim-milk powder, is compared with an iron-rich diet containing meat, as affecting the growth, the fasting metabolism, the blood composition, and the reproduction of the rat; and also as affecting the histology of the liver, the kidney, the spleen, the skeleton, and the muscle of this species.

REVIEW OF LITERATURE

Milk is a food having a characteristically low iron content. This was reported by Bunge (*10*).[3] Abderhalden (*1*) first showed that an animal fed an exclusive milk diet developed anemia. Hart, Steenbock, and associates (*22; 23; 24; 51; 11, p. 73–75*), Beard and his associates (*5, 6, 7, 8, 43, 44*), Titus and Hughes (*50*), and Krauss (*26, 27, 28, 29*) are among the recent investigators who have produced nutritional anemia by the exclusive feeding of liquid whole milk to rats. Whipple and Robscheit-Robbins (*52*) have demonstrated the ineffectiveness of milk to cause blood regeneration in dogs made anemic by bleeding, and Miller, Forbes, and Smythe (*40*), and Miller and Forbes (*39*) have shown that the iron content of the bodies of rats becomes subnormal as a result of feeding on diets in which the iron and protein is supplied solely by dried milk.

In spite of the fact rats fed milk are anemic, with less than normal amounts of iron in their bodies, the growth of rats on milk diets after weaning is not much less than the normal. Also during the suckling period the mother's milk supports rapid growth but does not induce much iron storage.

According to Abderhalden (*2*) and Williams and Ets (*53*), the percentage of hemoglobin in rat's blood diminishes during the suckling period, when the sole food is the mother's milk. Smythe and Miller (*49*) found a decrease in the percentage of total iron in the bodies of suckling rats, though the absolute amount of iron increased somewhat during the same period. After weaning, the percentage and absolute amounts of iron in the rats' bodies both increased rapidly. The iron content of females was depleted during pregnancy but

[1] Received for publication Nov. 3, 1932; issued June, 1933. This investigation was financed in part by a grant from the National Livestock and Meat Board fellowship fund of the National Research Council.
[2] The authors wish to express their appreciation to Dr. E. B. Forbes for the initiation and direction of this investigation; to Prof. G. B. Newman, of the Pennsylvania State College, for guidance in the histological work; and to Dr. Peter Olafson, of the New York State Veterinary College, at Cornell University, for counsel in the interpretation of conditions presented in the preparation of the tissues.
[3] Reference is made by number (italic) to Literature Cited, p. 959.

returned to normal after the young were weaned. These observations of Smythe and Miller on suckling rats have been confirmed by Lintzel and Radeff (*30*).

It is recognized, on the strength of evidence to be cited, that exclusive milk diets do not adequately support reproduction, and there is some evidence that the low iron content of milk is, at least in part, responsible for its nutritive deficiency in this respect.

Waddell, Steenbock, and Hart (*11*) found that when milk was fed to rats as the sole food, fortified only with copper and iron, living young were produced to the third generation, although both growth and reproduction were diminished. When small amounts of manganese, or iodine, or both, were added to the diet reproduction improved greatly.

Krauss (*29*) did not obtain any reproduction when rats were fed an exclusive whole milk diet. The addition of copper; iron and copper; iron, copper, and yeast; iron, copper, and wheat-germ oil; yeast alone; or cane molasses permitted reproduction, but this was in no case normal. Lactation was poor.

Daniels and Hutton (*14*) overcame the reproductive difficulty accompanying exclusive milk feeding by using a complex mineral supplement containing aluminum, potassium, sodium, fluorine, silicon, manganese, iron, and iodine.

Anderegg (*3*) obtained reproduction and lactation while feeding whole milk powder with supplements of iron citrate, 0.2 per cent, and agar-agar, 4.8 per cent. Later Anderegg and Nelson (*4*) made a study to determine the supplements necessary to make whole and skim milk powders adequate to support reproduction. Whole-milk powder properly supplemented with iron salts and carbohydrate, or with iron salts alone, supported good growth, reproduction, and rearing of young, the amount of iron salts added having a pronounced effect upon the reproduction. Skim-milk powder required an additional supplement of either yeast or wheat embyro before successful reproduction could be obtained.

Clayton (*12*) credits part of the success of Anderegg to the ash content of the agar-agar.

Mattill and Conklin (*35*) observed that when weanling rats were placed on a liquid milk diet no reproduction occurred, even though good growth resulted for 50 or 100 days; neither did the use of dried whole milk or dried skim milk, to which butter was added, favorably influence reproduction. Examination of the testes showed that motile spermatozoa were present. The ovaries were 50 per cent below normal weight. The authors stated that possibly milk is lacking both quantitatively and qualitatively in substances necessary for successful adolescent growth and for reproduction, especially in the female. Later Mattill and Stone (*36*) found reproduction unsuccessful in both male and female rats receiving rations in which all of the protein and known vitamins were supplied by varying proportions of dried whole milk.

Still later Mattill, Carman, and Clayton (*34*) found that a milk diet high in fat did not support reproduction in rats, but that the addition of 5 to 10 per cent of wheat embyro restored reproductive powers. When a milk diet low in fat was used, marked reproductive failure did not appear.

Sherman and Muhlfeld (*48*) demonstrated that as the proportion of whole-milk powder was increased in a diet already adequate for growth, reproduction, and lactation, there resulted increased fertility among females, an increase in the number of offspring, an increase in the size attained by the young during suckling, and a longer period of reproduction. These findings, therefore, show that while milk used as the sole food is nutritively adequate to support growth, its deficiency in iron, and in other factors, results in deficient nutrition of the blood, and possibly also in subnormal reproduction.

Meat as compared with milk has a relatively high iron content, and its usefulness in the nutrition of the blood and in curing secondary anemias is well established. The literature concerning iron in this relation has been reviewed by Hall (*20*), Meyer (*38*), Robscheit-Robbins (*47*), and Krauss (*29*).

Miller and Forbes (*39*) have reviewed the literature pertaining to the value of meat protein for growth, and for reproduction.

The present investigation was concerned with the relative usefulness of meat and milk as sources of iron for growth, nutrition of the blood, and reproduction, and as affecting heat production and the histology of the tissues.

EXPERIMENTAL PROCEDURE

An outline of the schedule of experimentation is presented in Table 1. The experimental subjects were albino rats from the colony of the Institute of Animal Nutrition of the Pennsylvania State College.

Experimental feeding began immediately after weaning, at which time the rats were 24 to 28 days old and weighed from 35 to 45 g. These rats were the offspring of females that had been fed the stock diet, the composition of which is given in the following tabulation:

Stock ration:	Parts
Yellow corn	25
Wheat red-dog flour	22
Oat flour	15
Linseed meal	15
Blood flour	10
Ground malted barley	10
Sodium chloride	1
Precipitated calcium carbonate	1
Precipitated bone	1
Cod-liver oil	5
Wheat-germ middlings	5
Total	110

This ration contained 22 per cent of crude protein and 0.027 per cent iron. Part of the stock from which the rats were derived had been fed this diet during the last 14 generations. In addition, wheat-germ oil was given to the females once daily, with a medicine dropper, during the latter half of the growth period and throughout the periods of breeding and pregnancy.

The experimental rats were fed individually in galvanized-wire cages provided with wire false bottoms to prevent access to excreta. Each rat had access to fresh tap water at all times.

The food intake was controlled in all animals, except the females during gestation and lactation, when they were allowed as much food as they would consume; otherwise all rats were fed weighed daily portions of feed, seven such portions being weighed at one time and stored in tin boxes until used.

TABLE 1.—*Schedule of experimentation*

Generation of animals, experimental series, and date	Diet No.	Number and sex of rats	Duration of experimental feeding	Protein feeds	Experimental treatment
First generation:					
Series 1— Started Oct. 19, 1928	23	4 females, 4 males	*Weeks* 15	Skim-milk powder	All animals used for the determination of heat production and blood constituents; 4 females used for reproduction; 4 males used for histological investigation.
	24	4 females, 4 males	15	Skim-milk powder and beef muscle.	Do.
Series 2— Started Mar. 23, 1929	23	5 females	15	Skim-milk powder	Used for testing reproduction.
	24	do	15	Skim-milk powder and beef muscle.	Do.
Series 3— Started Jan., 1930	23	15 females	15	Skim-milk powder	Used for testing reproduction and blood constituents.
	24	16 females	15	Skim-milk powder and beef muscle.	Do.
Second generation: Females from series 1, 2, and 3	23	11 females	15	Skim-milk powder	Do.
	24	18 females	15	Skim-milk powder and beef muscle.	Do.

The rats received the vitamin supplements separately, pellets of Harris Yeast Vitamin being given daily in the feed cups, and cod-liver oil daily with a medicine dropper.

For the growth experiments the rate of food allowance was determined by the food consumption of the group of rats eating the smaller amount, the quantity of food eaten by this lot, in relation to its live weight, constituting the bases for the allowance of feed to the other lot. The quantities of food thus assigned are presented in Table 2, the make-up of the rations and the chemical composition of the diets in Table 3. The experience gained from this method of feeding, in the experiments of series 1 of the first generation, made possible the more successful regulation of the food in the succeeding experiments with both generations. The rats were weighed at weekly intervals, and the quantity of food given was computed on the basis of these weekly weights.

TABLE 2.—*Numerical basis of control of food intake in grams eaten per week per gram of body weight*

Week No.	First generation						Second generation	
	Series 1		Series 2		Series 3		From first-generation series 1, 2, and 3	
	Diet No. 23	Diet No. 24	Diet No. 23	Diet No. 24	Diet No. 23	Diet No. 24	Diet No. 23	Diet No. 24
1	0.62	0.65	0.84	0.78	0.78	0.82	0.76	0.72
2	.69	.70	.64	.63	.66	.64	.61	.64
3	.70	.59	.55	.56	.57	.59	.57	.57
4	.50	.54	.53	.49	.49	.51	.53	.51
5	.47	.46	.48	.48	.49	.48	.49	.47
6	.43	.41	.42	.42	.43	.42	.41	.44
7	.39	.38	.39	.38	.39	.39	.38	.39
8	.39	.34	.35	.38	.35	.37	.35	.38
9	.34	.31	.33	.34	.32	.32	.31	.32
10	.32	.30	.31	.31	.31	.31	.29	.31
11	.30	.30	.30	.30	.30	.30	.29	.30
12	.28	.29	.29	.28	.28	.29	.28	.29
13	.29	.27	.27	.27	.27	.27	.27	.27
14	.26	.28	.25	.25	.25	.25	.25	.25
15	.25	.24	.25	.25	.25	.25	.25	.25

TABLE 3.—*Composition of diets* [a]

Components and constituents of diets	Diet No. 23[b]	Diet No. 24[c]
Components:	*Per cent*	*Per cent*
Skim-milk powder	44.37	44.37
Dried beef muscle		20.00
Lactose	10.00	10.00
Starch	31.95	21.03
Crisco	13.18	4.10
Sodium chloride	.50	.50
Fat	13.60	10.00
Constituents:		
Crude protein	15.00	27.00
Iron	.001	.002

[a] Cod-liver oil and Harris Yeast Vitamin were given daily, apart from the ration.
[b] Energy value, 494.7 calories per kilogram.
[c] Energy value, 494.5 calories per kilogram.

When this method of food control is used, the apparent palatability of the diet, which serves to determine the rate of feeding, may be fundamental, in the sense of being truly characteristic, or may be a secondary result of nutritive deficiency and consequent low state of nutrition. In this investigation the quantity of food consumed in proportion to the body weight became smaller each week until the maximum body weight was attained.

There is room for question, however, as to the equitableness of this method of feed apportionment. Thus, since the maintenance requirement of the smaller animal is greater in proportion to body weight than is that of the larger one, a smaller quantity of food in proportion to body weight is left for gain. In this respect, the larger animal is at a comparative advantage; but in another the advantage may rest with the smaller animal, which has thriven less well, since it may have less desire for food than has the larger animal, which has made the better growth. The larger animal, therefore, perhaps suffers from an unsatisfied appetite whereas the smaller animal does not. Since the comparative reaction of the two animals may differ with the nature of the ration it is impossible to say, in general terms, which animal enjoys the net advantage.

At the termination of the 15 weeks feeding for growth, the rats were used for other phases of the study.

The beef muscle fed was purchased in the local market. The lean portion was freed from the bone and fat and was ground twice in a food chopper made of nearly iron-free phosphor-bronze. The finely ground fresh muscle was placed in a glass drying dish and dried in a Freas oven at about 85° C. The dried material was then further ground in the phosphor-bronze chopper.

In planning the feeding treatments the iron-poor diet (No. 23) was first adopted, dried skim milk being the sole source of protein and iron; then the iron-rich diet (No. 24) was devised by substituting dried beef muscle for an equi-caloric portion of the nonnitrogenous components of the iron-poor diet. The two diets, therefore, were balanced as to energy value, but not as to protein content. The diets were not balanced as to protein because the protein of the iron-poor diet (dried skim milk) was considered to be fully adequate, both as to quantity and composition, and it would be theoretically impossible for the added dried beef muscle either to augment or to diminish the protein value of the diet. Also inasmuch as vitamin and mineral

nutrient requirements (other than iron) were fully satisfied by both diets, the basis was established for the demonstration of the additional nutrititive value of diet 24, as compared with diet 23, presumably on account of its higher iron content, but possibly on account of other constituents of the meat.

The iron content of the diets was determined by the modified thiocyanate-acetone method, which is discussed in detail by Miller, Forbes, and Smythe (*40*).

GROWTH

The data representing food intake and body gain are given in Table 4, while curves representing average growth, and histographs of total weekly food consumption are given in Figure 1.

Approximately normal growth was obtained from both diets throughout the three series, but the rats fed the high-iron diet (No. 24) made a slightly greater increase in body weight in each case. This difference, however, is too small to be significant. In series 1, 2, and 3 the average daily gains in body weight of the rats which received the high-iron diet (No. 24) were on the average slightly greater than the gains of the rats which received the low-iron diet (No. 23). Miller and Forbes (*39*) in an experiment similar to series 1 fed the same two diets at a lower rate of food intake and obtained a slightly greater average daily gain in body weight with the group fed the low-iron diet.

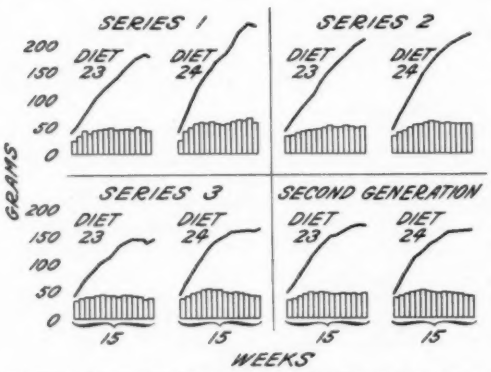

FIGURE 1.—Average growth (curves) and average food intake (bars) of rats on iron-deficient (No. 23) and high-iron (No. 24) diets

The food consumpti n per gram of body weight was practically the same for the rats of both groups in all experiments; and the iron intake of the rats fed the high-iron diet was more than twice that of those fed the low-iron diet.

The findings of all three series are similar in respect to growth. The composite curves in Figure 1 show that the average rate of gain was lower for the rats of series 3 (first generation) and for the rats of the second generation than the average rates of gain for the rats of series 1 and 2 (first generation), which may possibly have been the result of the very warm weather which prevailed during the feeding of the groups first mentioned. This suggestion is supported by the experience of Hanson and Hays (*21*) who found, in a study of the growth of rats during different seasons of the year, that females born during the fall have the greatest mean body weight at all ages and that those born during the summer have the smallest body weight.

TABLE 4.—*Foods and iron consumed and gains in body weight*

Generation and number of animals and experimental series	Sex	Diet No.	Source of protein and iron	Average daily gain in body weight	Average daily food intake	Average daily iron intake	Average weekly food intake per gram of body weight
				Grams	*Grams*	*Milligram*	*Gram*
First generation:							
Series 1—							
4	Male	23	Skim-milk powder	1.48±0.09	6.49	0.07	0.42
4	Female	23	Skim-milk powder	1.17± .03	6.04	.06	.42
4	Male	24	Skim-milk powder and beef muscle.	2.22± .07	8.75	.18	.41
4	Female	24	Skim-milk powder and beef muscle.	1.43± .07	6.82	.14	.40
Series 2—							
5	do	23	Skim-milk powder	1.57± .06	6.46	.07	.41
5	do	24	Skim-milk powder and beef muscle.	1.68± .07	7.26	.14	.41
Series 3—							
15	do	23	Skim-milk powder	.98± .04	5.53	.06	.41
16	do	24	Skim-milk powder and beef muscle.	1.15± .03	6.49	.13	.42
Second generation—							
11	do	23	Skim-milk powder	1.20± .04	5.91	.06	.40
17	do	24	Skim-milk powder and beef muscle.	1.13± .03	6.32	.13	.42

The food consumption and the average daily gains in body weight of the second-generation females are presented in Table 4 and in Figure 1. Since the number of individuals per treatment in each series of comparison was diminished in the tests of the second generation, as compared with the first, especially among the individuals fed the low-iron diet, the individuals of the three series on each diet were combined to determine the average food consumption and gain in body weight.

No evidence is found in the growth data to indicate that the low-iron diet adversely influenced the gain in body weight during two generations of feeding.

HEAT PRODUCTION

One phase of the investigation was to determine the effect of previous feeding with the low-iron and high-iron diets on the fasting heat production.

Meyer and DuBois (*37*) found an increased demand for oxygen in severe anemia, but no conclusive evidence was found of a definite level of basal metabolism characteristic of anemia.

Pettenkofer and Voit (*46*) made a study of a severe case of anemia in man, in which they found the metabolism to be exactly the same as for a normal resting man. This investigation, however, was reviewed by Magnus-Levy (*33*), who concluded that in this case metabolism was increased by anemia. He came to the same conclusion in a study of a severe case of anemia, conducted by himself.

In the present study each rat of the first series of tests was subjected to a measurement of the heat production by means of a modified Haldane apparatus (*19*).

In the conduct of a respiration experiment a rat was fasted for approximately 24 hours and was then kept for a period of 5 to 6 hours

in the respiration apparatus, during which time the carbon-dioxide production and the oxygen consumption were determined. The heat production as computed from these data, however, could not be considered accurately to represent the basal metabolism of the animal, since the activity of the subject was not controlled. In every experiment the animal spent the greater portion of the experimental period at complete rest, and in no instance was excessive activity observed.

The critical temperature of the rat, on a basal plane of nutrition, is about 28° C., according to Goto (*18*) and Benedict and MacLeod (*9*). The present respiration determinations were made during December, 1929, in a room with a fairly constant temperature, varying, however, between 26° and 30°. The rats, when not in the respiration chamber, were kept in the stock-colony room, which was maintained at a temperature of about 27° except during seasons when the normal temperature was above 27° .

The heat production was computed by the modified Zuntz and Schumburg method, as given by Lusk (*31*), using the total respiratory quotient, i. e., neglecting the katabolism of protein.

The details of the determinations of the heat production, together with information concerning sex, age, length of fast, body weight, and food consumption, are given in Table 5. The body surface areas of the rats were calculated from their body weights by means of the Meeh formula ($S = KW^{\frac{2}{3}}$), using the constant 7.47, recently derived by Diack (*15*). The age and sex of the subjects and the length of the periods of experimental feeding were approximately the same for the two groups. The heat production was determined at the eighth to the eleventh week of experimental feeding.

The average of the respiratory quotients, determined between 16 and 50 hours after the last feeding, was 0.75 (ranging between 0.72 and 0.77) for the low-iron diet (No. 23), and also 0.75 (ranging between 0.72 and 0.80) for the high-iron diet (No. 24).

The figures for the heat production of the rats in this study were calculated on two bases: (1) In relation to the two-thirds power of 100 g of body weight per 24 hours; and (2) per square meter of body surface per 24 hours. On the low-iron diet the average daily heat production of the rats was 15.56 calories per 100 g of body weight and 967 calories per square meter of body surface. For the high-iron diet the average daily heat production of the rats was 15.34 calories per 100 g of body weight, and 953 calories per square meter of body surface.

Benedict and MacLeod (*9*), using the constant 9.1 in the Meeh formula, found the average so-called basal metabolism of the albino rat, at 28° C. and above, to be about 775 calories per square meter of body surface per 24 hours, when no differentiation is made for sex. For females alone, the figure representing the basal metabolism at 28° and above is about 720 calories, and for males 800 calories.

Mitchell and Carman (*41*), using rats ranging in weight from 84 to 251 g, reported a fasting metabolism of 600 calories per square meter of body surface per 24 hours for males, and 571 calories for females (at complete rest), having used in the computation of the body surface the constant 11.36, in the Meeh formula.

If 7.47 instead of 9.1 is used as the constant in the surface-area formula, the values of Benedict and MacLeod are 944, instead of 775 calories, where sex is not considered, and 877 calories for females,

and 975 calories for males. The figures of Mitchell and Carman, when recomputed in the same way, are 912 and 868 calories for males and females, respectively.

The average values obtained in this investigation, for the fasting heat production per square meter of body surface per 24 hours are in close agreement with values reported by Benedict and MacLeod (*9*) and are higher than those obtained by Mitchell and Carman (*41*). A slightly higher value should be expected in this investigation since the data were taken from rats during periods of five or six hours, with no control over activity, while the above-mentioned investigators obtained values from rats during short periods of complete rest.

The high-iron and low-iron diets, however, did not differ in their effect on subsequent fasting heat production, and the results as given in Table 5 are all in good agreement with those representing normal individuals as reported by other investigators.

TABLE 5.—*Data relating to the determination of the heat production of rats on low- and high-iron diets*

DIET 23, LOW IRON

Rat No.	Sex	Age	Length of fast	Body weight	Respiration period	Respiratory quotient	Oxygen consumed per day	Heat produced per day		
								Total	Per 100 g body weight[a]	Per square meter of body surface
		Days	*Hours*	*Grams*	*Minutes*		*Liters*	*Calories*	*Calories*	*Calories*
508	Female	97	20	135	240	0.75	3.87	18.34	15.01	933
509	Male	86	20	134	973	.75	4.40	20.85	17.15	1,066
510	do	89	29	125	994	.74	3.66	17.30	14.91	927
511	Female	95	24	132	300	.77	3.41	16.25	13.50	839
512	Male	97	24	171	300	.74	4.77	22.55	15.76	979
513	Female	98	19	149	353	.72	4.81	22.62	17.34	1,078
514	Male	80	16	141	300	.76	3.91	18.58	14.78	918
515	Female	91	24	136	300	.77	4.13	19.68	16.04	997
Average		91				.75			15.56	967

DIET 24, HIGH IRON

500	Female	85	25	141	360	0.80	3.37	16.18	12.86	799
500	do	86	50	135	300	.78	3.61	17.24	14.11	877
501	Male	88	22	192	280	.72	5.52	25.96	16.80	1,044
502	Female	96	25	188	285	.73	5.23	24.65	16.18	1,005
503	do	93	27	151	300	.75	4.00	18.96	14.41	895
504	Male	97	20	205	290	.78	5.06	24.17	14.97	930
505	do	97	22	237	300	.74	5.95	28.13	15.82	982
506	do	95	27	203	300	.74	5.47	25.86	16.13	1,002
507	Female	85	22	147	300	.73	4.59	21.64	16.74	1,040
Average		91				.75			15.34	953

[a] Computed to correspond to uniform weight, from the total heat production in relation to the two-thirds powers of the body weight.

BLOOD CONSTITUENTS

The quantity of hemoglobin in the blood was determined, and red and white blood cell counts were made on the animals of series 1 and 3 of the first generation, and also on the rats of the second generation.

Waddell (*11*) dispensed with the erythrocyte count on the ground that it added little information to that obtained from hemoglobin

determinations and involved considerable extra labor. However, Beard and his associates (*7*, *43*) emphasized the importance of making erythrocyte counts in addition to hemoglobin estimations, inasmuch as erythropoietic stimulants seem to act first on red blood-cell formation.

Although Jencks (*25*), Waddell (*51*), Mitchell (*42*), and McCay (*32*) successfully obtained blood from the tail of a rat, the writers found it easier to obtain it from a vein (the saphena parva) of the thigh. More blood is obtainable from this vein in a given time than from the tail, and there is less disturbance of the animal.

The procedure used in drawing the blood sample was as follows: The hair is clipped close to the skin of the outside of the thigh just above the knee joint. The place to be punctured is scraped carefully with a sharp knife and then washed and dried. The scraping and

FIGURE 2.—Method of holding rat and securing blood sample from the vena saphena parva

washing are done to remove from the skin substances which hasten the clotting of the blood. Pressure is exerted with a thumb and forefinger, about the hip joint, to increase the blood in the vena saphena parva. This vein is then punctured with a blood lancet, just above the knee joint. It is possible to obtain with one puncture enough blood for the hemoglobin determination and the erythrocyte and leucocyte counts. The method is illustrated in Figure 2.

The samples for determination of blood constituents were taken during the week succeeding the termination of the growth-feeding period. Hemoglobin estimations were made by the acid-hematin method (*13*), color comparisons being made with a calibrated Newcomer disk (*45*). A standard counting chamber with the improved Neubauer ruling was used for the erythrocyte and leucocyte counts. The erythrocytes were diluted for counting (1:200) in Hayem's solution (composed of 200 cc of distilled water, 1 g of sodium chloride,

5 g of sodium sulphate, 0.5 g mercuric chloride). The leucocytes were diluted for counting (1:20) in a solution of 1 per cent glacial acetic acid tinged with gentian violet.

The rats on both diets were normal with respect to blood constituents as shown in Table 6.

TABLE 6.—*Blood constituents of rats on low-(No. 23) and high-(No. 24) iron diets*

Diet No.	Generation	Hemoglobin		Erythrocytes		Leucocytes	
		Rats	Average per 100 cc of blood	Rats	Average per cubic millimeter of blood	Rats	Average per cubic millimeter of blood
		Number	*Grams*	*Number*	*Number*	*Number*	*Number*
24	First	24	17.44±0.25	24	8,175,000±111,000	21	7,500±140
23	do	24	15.37± .13	23	7,772,000±148,000	21	7,560±122
24	Second	13	16.89± .32	10	9,787,000±205,000	8	7,420±216
23	do	10	15.95± .28	5	7,874,000±156,000	4	7,950±506

In the first generation the average hemoglobin content of the blood of the rats on the high-iron diet was 17.44 g per 100 cc of blood, as compared to 15.37 g for the rats on the low-iron diet. The difference, 2.07 ± 0.28 g, is statistically significant. In the second generation, the hemoglobin content of the blood of the rats on the two diets was not significantly different from that of the rats of the first generation, and although it was slightly higher for the rats on the high-iron diet as compared to that for the rats on the low-iron diet, the difference was not significant. All the hemoglobin values may be considered normal.

The average number of erythrocytes per cubic millimeter of blood for the rats in the first generation on the high-iron diet was 8,175,000 and for those on the low-iron diet 7,772,000; for the second generation 9,787,000 for those on the high-iron diet and 7,874,000 for those on the low-iron diet. The number of erythrocytes is significantly higher for the rats fed the high-iron diet in the second generation as compared with first generation rats on the same diet, and with first and second generation rats on the low-iron diet. The results with respect to the number of leucocytes in the blood show no significant differences in any of the treatments.

Miller and Forbes (*39*), feeding the same diets used in this investigation, found that rats fed the high-iron diet (No. 24) stored more iron in the body than did those given the low-iron diet (No. 23), but this difference in total iron stored was much more pronounced than were the differences in hemoglobin and in blood cells as observed in the present study. This fact is significant in relation to the observation of McCay (*32, p. 29*).

A decrease in the rate of recovery during a long anemic period shows that the usual criteria of blood regeneration may be false. When the cell count and hemoglobin of an anemic animal have returned to normal it is said to be recovered. The recovery is probably only apparent in many cases since the reserves may remain very low.

REPRODUCTION

After the termination of the 15-week growth experiment, the female rats from series 1, 2, and 3, the treatment of which was indicated in Table 1, became the subjects of a study of the effects of iron deficiency on reproduction. The individuals of each category as to iron content of ration, and generation of young produced, were considered as a separate group in this breeding experiment, there being four such groups. A summary statement of the results of the four groups is given in Table 7.

TABLE 7.—*Summary of results relating to reproduction and lactation of rats on low (No. 23) and high (No. 24) iron diets*

Generation and diet No.	Female experimental subjects	Females that did not reproduce	Total young born	Young born dead	Young that died during suckling period	Young weaned		Average weight of young at birth	Average young per litter at birth	Average weight of young at weaning	Average young per litter at weaning
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Grams*	*Number*	*Grams*	*Number*
First generation:											
23	25	8	117	8	33	76	65.0	4.9	6.9	42.1	5.8
24	26	6	168	0	22	142	84.5	5.0	8.4	40.5	7.5
Second generation:											
23	11	1	[a] 69	15	30	24	34.8	[b] 4.6	[a] 6.9	36.2	6.0
24	18	2	133	3	21	105	79.0	4.7	8.3	44.3	6.6

[a] Includes 6 atrophic, partly developed fetuses.
[b] Does not include 6 partly developed fetuses.

The females were mated as soon as possible after the termination of the growth experiment, and each was kept in an individual cage in which she was fed until her young were born and then weaned, 25 females being given the low-iron and 26 the high-iron diet. A daily supplement of wheat-germ oil was fed with both diets to supply vitamin E during the time of the study of reproduction.

In the study of first-generation reproduction, 117 young were produced by 17 females receiving the low-iron diet (No. 23), and 168 young by 20 females receiving the high-iron diet (No. 24). Six females on the high-iron diet and 8 females on the low-iron diet did not produce any young, 1 female in each group having died while pregnant, and the others having failed to become pregnant during at least seven weeks to males previously proved to be fertile. The latter females were removed from the experimental groups and discarded.

The young produced on both diets weighed about the same at birth; but at the time of weaning the average weight of the young whose mothers were fed the low-iron diet was 1.6 g more than that of those whose mothers received the high-iron diet, this difference probably depending on the smaller number of young per litter from the females on the low-iron diet. Not only was the average number of young per litter at birth smaller for the low-iron group than for the high-iron group but so also was the percentage of young that survived during the suckling period. Four females on the low-iron diet failed to wean any young from a total of 27 born alive; while only one entire litter was lost during the suckling period by the females on the high-iron diet. Three of the females given the low-iron diet are known to have killed their young and in part to have eaten them. No dead

young were produced by the females on the high-iron diet, while 8 dead young were born to those on the low-iron diet.

Second-generation females from 11 mothers in the low-iron group and from 18 in the high-iron group were available for a further study of reproduction as affected by the two diets. (Table 7.)

Each second-generation female, from the time it was weaned until it had produced young and weaned them, was given the same diet as that which had been fed to its mother.

One female was discarded from the low-iron group because of failure to breed. Two females on the high-iron diet failed to breed, one of them having developed an abnormal growth of the incisor teeth, the cause of which was unknown. This condition appeared similar to that which develops when an excessive amount of fluorine is present in the diet; however, but one animal was affected, the teeth of the other members of the group appearing to be normal.

In the second generation, on both diets, approximately the same average number of young were produced per litter as in the first generation. The average weight of young at birth was approximately the same for both diets.

On the low-iron diet 34.8 per cent of the total number of young were weaned, 15 individuals having been born dead and 30 having died during the suckling period. In the first generation on this diet 65 per cent of all young were weaned. In both generations on the high-iron diet the females weaned a higher percentage of the total number of young than did the females on the low-iron diet.

While there was not much difference in the size of the rats weaned by females on both diets in the first generation, the average weight of those weaned by the females fed the high-iron diet in the second generation was considerably greater than that of the young weaned by the females of the low-iron group. Only 4 litters were weaned of the 10 produced by the latter group, and the size of the rats in 2 of these was considerably less than normal; on the other hand, 16 litters were produced and weaned by females on the high-iron diet in the second generation, and the weanling rats were normal in size.

Thirteen of the 17 females of the first generation which were given the low-iron diet (No. 23) weaned all or part of their young born. Five of 10 females of the second generation given the low-iron diet had a total of 15 young born dead, 1 female giving birth to 6 atrophic fetuses and 1 live but undersized individual. In the first generation, on the same diet, 4 out of 17 females had a total of 8 young born dead. Thirty young from 10 females of the second generation, fed the low-iron diet, died during the suckling period, as compared with 33 young from 17 females of the first generation fed the same diet.

In both generations the rats fed the low-iron diet had rough coats of fur and also seemed to be low in vitality, as indicated by lack of normal muscle tone. The inadequacy of the low-iron diet was also reflected in the appearance and behavior of the rats. In both generations some of these rats killed and ate a part of their young, but this did not occur in the case of the females on the high-iron diet in either generation.

A typical example of the effect of the iron-deficient diet on reproduction and lactation during the second generation is illustrated in Figure 3. The smaller rat was from a second-generation female on the low-iron diet and represents the average in size of the 8 rats of this

litter at 18 days of age. The larger rat was from a second-generation female on the high-iron diet and is the average in size of the rats of a litter of 14 young at 20 days of age.

It is apparent that the high-iron diet supported reproduction and lactation much more effectively than did the low-iron diet, and that marked nutritive deficiency characterized those rats which received the latter diet.

Since Miller and Forbes (*39*) found the total iron content of the bodies of rats fed the low-iron diet (No. 23) to be low, it is probable that in the present work the females which received this diet were likewise deficient in the iron content of their bodies, and that this deficiency was responsible for the observed lack of capacity for normally successful reproduction.

The unfavorable effects of iron deficiency on reproduction were more prominent in the performance of the second-generation females con-

FIGURE 3.—Representative second-generation rats, 20 days old, on high-iron diet, left; 18 days old, on low-iron diet, right

fined to a low-iron diet than in that of the first-generation females—which is understandable in the light of the following facts: The nourishment of the first-generation females on the low-iron diet began at the time of weaning; and the nourishment of the second-generation females on the low-iron diet began in the uterus of the mother at the time of conception. The first-generation females, therefore, were nourished during the periods of pregnancy and lactation by mothers supposedly normal with respect to the iron content of the diet and to the quantity of iron in their bodies, while the second-generation females were nourished by mothers that had been given an iron-deficient diet, beginning at the age of weaning, and were supposedly low in the iron content of the body. The deficiency of iron in the bodies of the second-generation females not only deprived the offspring of iron during embryonal development but also hindered the

normal functioning of the body of the mother during the periods of pregnacy and lactation.

From the performance of the rats while reproducing on the low-iron diet it appears that under this condition they will not sacrifice the iron of their own body tissues to such extent as to cause severe injury for the purpose of nourishing the young in utero. When the iron content of the body of a female is low the iron content of her embryos will be low, the exact quantities received determining whether the young will be absorbed from the uterus, or will be born immature, or will be developed to normal maturity.

HISTOLOGICAL STUDIES

A microscopic study was made of sections of the bone marrow, liver, kidney, spleen, and muscle tissue of the male rats of series 1. Since the formation of erythrocytes and hemoglobin involves these tissues, an iron deficiency, if sufficiently pronounced, would be expected to produce abnormalities in their structure.

Doyle, Mathews, and Whiting (*16*) made a microscopic study of the tissues of pigs which were severely anemic. The most prominent changes were marked fatty degenerative infiltration of the liver, and the presence of hematopoietic centers in the liver, spleen, and bone.

Drabkin and Miller (*17*) found no evidence of pathological lesions in the intestines of rats which had died from milk anemia.

The samples of tissues for histological study in the present investigation were obtained from the males of series 1 at the termination of the feeding trial. The animals were chloroformed, and the livers, kidneys, spleens, and certain bones and muscles were removed immediately, and placed in Diedrich's solution (composed of 12 cc of 40 per cent formalin, 30 cc of 95 per cent alcohol, 2 cc of glacial acetic acid, and 60 cc of distilled water). The liver, kidneys, spleens, and muscles were gradually dehydrated with alcohol solutions. After the drying process was completed, the samples were placed in celloidin for a period of six months. The bones, after dehydration, were treated with Von Ebner's fluid (500 cc of 95 per cent ethyl alcohol, 100 cc of water, 2.5 g sodium chloride, and 5.5 cc of concentrated hydrochloric acid). As soon as the bones were decalcified, they were placed in celloidin for a period of six months. Sections were cut 12μ in thickness and were stained with hematoxylin.

In an anemic animal morphological changes would naturally be sought in the hematopoietic tissues. Commonly these are fatty changes, and degeneration in the kidneys and liver, together with some necrosis. Hematopoietic centers appear in the liver and spleen, while fat tends to disappear from the bone marrow, being replaced by erythroblastic tissue.

All the tissues in this study appeared to be normal, and very similar for both groups of rats. In the spleens the amount of lymphoid tissue was about the same, and centers of hematopoiesis could be identified. In the bone marrow the fat content was about the same for both groups, and the cell types did not vary appreciably—thus indicating normal cellular activity.

These observations indicate that the low-iron diet did not differ in any marked respect from the meat diet in its ability to nourish the tissues involved in blood formation, and that any iron impoverishment caused by the lower intake of iron on this diet was not sufficiently

severe to cause pathological changes in these tissues in the course of 15 weeks' feeding.

SUMMARY AND CONCLUSIONS

A comparison was made of the effects of 2 diets—1 low in iron, and 1 high in iron—on the growth, the fasting metabolism, the blood composition, and the reproduction of the albino rat; and also on the histology of the liver, the kidney, the spleen, the skeleton, and the muscle of this species.

The iron-poor diet contained 0.001 per cent iron, the source of which was dried skim-milk powder. The iron-rich diet contained 0.002 per cent iron, the source of which was beef muscle and dried skim-milk powder.

An average daily intake of 0.06 mg of iron from skim-milk powder was found to be sufficient for normal growth. No significant difference was found in the average rate of growth of the experimental subjects on the two diets in either the first or the second generation.

The average of the respiratory quotients, after 16 to 50 hours without food, was 0.75 for the rats on both diets. The average daily heat produced was 15.56 and 15.34 calories per 100 g of body weight ($W^{2/3}$) and 967 and 953 calories per square meter of body surface ($S = KW^{2/3}$), on the low-iron and high-iron diets, respectively.

The average number of erythrocytes and of leucocytes per cubic millimeter of blood was approximately the same for rats confined to these diets during the first and also during the second generation.

The average number of grams of hemoglobin per 100 cc of blood was also normal on both diets for two generations of feeding. The rats receiving the high-iron diet during the first generation had an average of 2.07 g more of hemoglobin per 100 cc of blood than had those receiving the low-iron diet. This difference was found to be statistically significant, but the rats on the low-iron diet could not be said to be anemic.

It was shown that the low-iron diet was inadequate for the most successful reproduction, and that this effect was more prominent during the second than during the first generation. The females of the first generation gave birth to 6.9 and 8.4 young per litter, on the average, and weaned 65.0 and 84.5 per cent of the young while confined to the iron-poor and the iron-rich diets, respectively. The females of the second generation gave birth to 6.9 and 8.3 young per litter, on the average, and weaned 34.8 and 79.0 per cent of the young while confined to the iron-poor and the iron-rich diets, respectively.

Sterility and cannibalism were observed among the second generation females which received the low-iron diet.

No pathological conditions were found as to histology of the tissues of the liver, kidney, spleen, bone, and muscle that could be considered as effects of the low-iron diet.

When the protein required for growth was provided by skim-milk powder, in a diet which satisfied the energy and the vitamin requirements, normal growth resulted, even though the iron content of the diet was low; the nutrition of the bood, and of the tissues studied and the rate of energy metabolism were normal; but this low-iron diet did not satisfy the requirements for normal reproduction. These requirements were provided, however, by a similar diet containing additional iron as supplied by beef muscle.

LITERATURE CITED

(1) ABDERHALDEN, E.
1900. DIE RESORPTION DES EISENS, SEIN VERHALTEN IM ORGANISMUS UND SEINE AUSSCHEIDUNG. Ztschr. Biol. 39 : [113]–152.

(2) ———
1902. DAS VERHALTEN DES HÄMOGLOBIN WÄHREND DER SÄUGLINGSPERIODE. Hoppe-Seyler's Ztschr. Physiol. Chem. 34 : [500]–516.

(3) ANDEREGG, L. T.
1924. DIET IN RELATION TO REPRODUCTION AND REARING OF YOUNG. Jour. Biol. Chem. 59 : 587–599.

(4) ——— and NELSON, V. E.
1925. WHOLE AND SKIMMED MILK POWDERS AS FOOD. OBSERVATION ON A NEW VITAMIN FOR REPRODUCTION. Indus. and Engin. Chem. 17 : 451–455.

(5) BEARD, H. H.
1931. STUDIES IN THE NUTRITIONAL ANEMIA OF THE RAT. VI. THE EFFECT OF INORGANIC ELEMENTS UPON THE RATE OF BLOOD REGENERATION AND GROWTH. Jour. Biol. Chem. 94 : 135–146, illus.

(6) ——— BAKER, R. W., and MYERS, V. C.
1931. STUDIES IN THE NUTRITIONAL ANEMIA OF THE RAT. V. THE ACTION OF IRON AND IRON SUPPLEMENTED WITH OTHER ELEMENTS UPON THE DAILY RETICULOCYTE, ERYTHROCYTE, AND HEMOGLOBIN RESPONSE. Jour. Biol. Chem. 94 : 123–134, illus.

(7) ——— and MYERS, V. C.
1931. STUDIES IN THE NUTRITIONAL ANEMIA OF THE RAT. I. INFLUENCE OF IRON UPON BLOOD REGENERATION. Jour. Biol. Chem. 94 : 71–88.

(8) ——— RAFFERTY, C., and MYERS, V. C.
1931. STUDIES IN THE NUTRITIONAL ANEMIA OF THE RAT. III. THE PREVENTION OF ANEMIA BY MEANS OF INORGANIC ELEMENTS. Jour. Biol. Chem. 94 : 111–115.

(9) BENEDICT, F. G., and MACLEOD, G.
1929. THE HEAT PRODUCTION OF THE ALBINO RAT. II. INFLUENCE OF ENVIRONMENTAL TEMPERATURE, AGE, AND SEX; COMPARISON WITH THE BASAL METABOLISM OF MAN. Jour. Nutrition 1 : 367–398, illus.

(10) BUNGE, G.
1899. UEBER DIE AUFNAHME DES EISENS IN DEN ORGANISMUS DES SÄUGLINGS. Hoppe-Seyler's Ztschr. Physiol. Chem. 13 : [399]–406.

(11) WADDELL, J., STEENBOCK, H., and HART, E. B.
1931. LABORATORY STUDIES SHOW THE WAY IN ANIMAL NUTRITION. Wis. Agr. Expt. Sta. Bul. 420 : 73–84, illus.

(12) CLAYTON, M. M.
1930. THE COMPARATIVE VALUE OF DIFFERENT FOOD PROTIENS FOR REPRODUCTION AND LACTATION IN THE RAT. II. MILK, EGG, AND MEATS. Jour. Nutrition 3 : 23–38.

(13) COHEN, B., and SMITH, A. H.
1919. THE COLORIMETRIC DETERMINATION OF HEMOGLOBIN. A PRACTICAL PROCEDURE. Jour. Biol. Chem. 39 : 489–496.

(14) DANIELS, A. L., and HUTTON, M. K.
1925. MINERAL DEFICIENCIES OF MILK AS SHOWN BY GROWTH AND FERTILITY OF WHITE RATS. Jour. Biol. Chem. 63 : 143–156, illus.

(15) DIACK, S. L.
1930. THE DETERMINATION OF THE SURFACE AREA OF THE WHITE RAT. Jour. Nutrition 3 : 289–296.

(16) DOYLE, L. P., MATHEWS, F. P., and WHITING, R. A.
1927. ANEMIA IN YOUNG PIGS. Ind. Agr. Expt. Sta. Bul. 313, 18 p., illus. (Also Jour. Amer. Vet. Med. Assoc. 72 : 491–510, illus., 1928.)

(17) DRABKIN, D. L., and MILLER, H. K.
1931. HEMOGLOBIN PRODUCTION. II. THE RELIEF OF ANEMIA, DUE TO MILK DIET, BY FEEDING AMINO ACIDS. Jour. Biol. Chem. 90 : 531–543, illus.

(18) GOTO, K.
1923. BEITRAG ZUR KENNTNIS DER CHEMISCHEN WÄRMEREGULATION DER SÄUGETIERE. III. WÄRMEREGULATION DER WEISSEN RATTE. Biochem. Ztschr. 135 : [107]–121.

(19) HALDANE, J.
1892. A NEW FORM OF APPARATUS FOR MEASURING THE RESPIRATORY EXCHANGE OF ANIMALS. Jour. Physiol. 13 : [419]–430, illus.
(20) HALL, W. S.
1894. UEBER DIE RESORPTION DES CARNIFERRINS. Arch. Anat. Physiol. 18 : 455–490, illus.
(21) HANSON, F. B., and HEYS, F.
1927. DIFFERENCES IN THE GROWTH CURVES OF ALBINO RATS BORN DURING THE FOUR SEASONS OF THE YEAR UNDER UNIFORM LABORATORY CONDITIONS. Anat. Rec. 35 : 83–89, illus.
(22) HART, E. B., ELVEHJEM, C. A., WADDELL, J., and HERRIN, R. C.
1927. IRON IN NUTRITION. IV. NUTRITIONAL ANEMIA ON WHOLE MILK DIETS AND ITS CORRECTION WITH THE ASH OF CERTAIN PLANT AND ANIMAL TISSUES OR WITH SOLUBLE IRON SALTS. Jour. Biol. Chem. 72 : 299–320, illus.
(23) ——— STEENBOCK, H., ELVEHJEM, C. A., and WADDELL, J.
1925. IRON IN NUTRITION. I. NUTRITIONAL ANEMIA ON WHOLE MILK DIETS AND THE UTILIZATION OF INORGANIC IRON IN HEMOGLOBIN BUILDING. Jour. Biol. Chem. 65 : 67–80.
(24) ——— STEENBOCK, H., WADDELL, J., and ELVEHJEM, C. A., with the cooperation of VAN DONK, E., and RIISING, B. M.
1928. IRON IN NUTRITION. VII. COPPER AS A SUPPLEMENT TO IRON FOR HEMOGLOBIN BUILDING IN THE RAT. Jour. Biol. Chem. 77 : 797–812, illus.
(25) JENCKS, Z.
1922. STUDIES IN THE REGENERATION OF BLOOD. Amer. Jour. Physiol. 59 : 240–253, illus.
(26) KRAUSS, W. E.
1929. STUDIES ON THE NUTRITIVE VALUE OF MILK. I. THE PRODUCTION OF NUTRITIONAL ANEMIA IN ALBINO RATS THROUGH EXCLUSIVE WHOLE MILK DIETS. Jour. Dairy Sci. 12 : 74–79, illus.
(27) ———
1929. STUDIES ON THE NUTRITIVE VALUE OF MILK. II. THE SUPPLEMENTARY VALUE OF INORGANIC IRON AND COPPER. Jour. Dairy Sci. 12 : 242–251, illus.
(28) ———
1929. STUDIES ON THE NUTRITIVE VALUE OF MILK. III. THE SUPPLEMENTARY VALUE OF VARIOUS CONSTITUENTS OF SYNTHETIC BASAL RATIONS. Jour. Dairy Sci. 12 : 438–444.
(29) ———
1931. STUDIES ON THE NUTRITIVE VALUE OF MILK. I. THE DEFICIENCIES OF AN EXCLUSIVE MILK DIET AND HOW TO OVERCOME THEM. Ohio Agr. Expt. Sta. Bul. 477, 54 p., illus.
(30) LINTZEL, W., and RADEFF, T.
1931. UEBER DEN EISENGEHALT UND EISENANSATZ NEUGEBORENER UND SAUGENDER TIERE (NACH VERSUCHEN AN KANINCHEN, MEERSCHWEINCHEN, RATZE, HUND, KATZE, SCHWEIN, ZIEGE, RIND). Wiss. Arch. Landw., Abt. B., Arch. Tierernährung u. Tierzucht. 6 : 313–358.
(31) LUSK, G.
1928. THE ELEMENTS OF THE SCIENCE OF NUTRITION. Ed. 4, reset, 844 p., illus. Philadelphia and London.
(32) MCCAY, C. M.
1928. THE INFLUENCE OF PROTEIN, BLOOD, LIVER, FAT, IRON AND POTASSIUM IN THE DIET UPON THE RATE OF BLOOD REGENERATION AFTER HEMORRHAGE IN THE RAT AND DOG. Amer. Jour. Physiol. 84 : 16–35.
(33) MAGNUS-LEVY, A.
1906. DER EINFLUSS VON KRANKHEITEN AUF DEN ENERGIEHAUSHALT IM RUHEZUSTAND. Ztschr. Klin. Med. 60 : [177]–224.
(34) MATTILL, H. A., CARMAN, J. S., and CLAYTON, M. M.
1924. THE NUTRITIVE PROPERTIES OF MILK. III. THE EFFECTIVENESS OF THE X SUBSTANCE IN PREVENTING STERILITY IN RATS ON MILK RATIONS HIGH IN FAT. Jour. Biol. Chem. 61 : 729–740, illus.
(35) ——— and CONKLIN, R. E.
1920. THE NUTRITIVE PROPERTIES OF MILK, WITH SPECIAL REFERENCE TO REPRODUCTION IN THE ALBINO RAT. Jour. Biol. Chem. 44 : 137–158, illus.

(36) MATTILL, H. A., and STONE, N. C.
1923. THE NUTRITIVE PROPERTIES OF MILK WITH SPECIAL REFERENCE TO REPRODUCTION IN THE ALBINO RAT. II. Jour. Biol. Chem. 55 : 443–455, illus.

(37) MEYER, A. L., and DUBOIS, E. F.
1916. THE BASAL METABOLISM IN PERNICIOUS ANEMIA. Arch. Int. Med. 17 : [965]–979.

(38) MEYER, E.
1906. ÜBER DIE RESORPTION UND AUSSCHEIDUNG DES EISENS. Ergeb. Physiol. 5 : [698]–745.

(39) MILLER, R. C., and FORBES, E. B.
1931. THE UTILIZATION OF THE IRON OF PROTEIN FOODS BY THE ALBINO RAT A (A) COMPARISON OF THE GROWTH AND THE IRON ASSIMILATION AS AFFECTED BY DIFFERENT PROTEIN FOODS: (B) A COMPARISON OF PROTEIN FOODS SUPPLEMENTARY TO MILK AS SOURCES OF IRON IN NUTRITION. Jour. Nutrition 4 : 485–505.

(40) ——— FORBES, E. B., and SMYTHE, C. V.
1929. A STUDY OF THE UTILIZATION OF THE IRON OF MEATS AS COMPARED WITH OTHER PROTEIN FOODS. Jour. Nutrition 1 : 217–232.

(41) MITCHELL, H. H., and CARMAN, G. G.
1926. EFFECT OF EXCESSIVE AMOUNTS OF VITAMIN B ON THE BASAL METABOLISM OF RATS OF DIFFERENT AGES. Amer. Jour. Physiol. 76 : 385–397, illus.

(42) MITCHELL, H. S., and SCHMIDT, L.
1926. THE RELATION OF IRON FROM VARIOUS SOURCES TO NUTRITIONAL ANEMIA. Jour. Biol. Chem. 70 : 471–486, illus.

(43) MYERS, V. C., and BEARD, H. H.
1931. STUDIES IN THE NUTRITIONAL ANEMIA OF THE RAT. II. INFLUENCE OF IRON PLUS SUPPLEMENTS OF OTHER INORGANIC ELEMENTS UPON BLOOD REGENERATION. Jour. Biol. Chem. 94 : 89–110.

(44) ——— BEARD, H. H., and BARNES, B. O.
1931. STUDIES IN THE NUTRITIONAL ANEMIA OF THE RAT. IV. THE PRODUCTION OF HEMOGLOBINEMIA AND POLYCYTHEMIA IN NORMAL ANIMALS BY MEANS OF INORGANIC ELEMENTS. Jour. Biol. Chem. 94 : 117–122.

(45) NEWCOMER, H. S.
1919. ABSORPTION SPECTRA OF ACID HEMATIN, OXYHEMOGLOBIN, AND CARBON MONOXIDE HEMOGLOBIN. A NEW HEMOGLOBINOMETER Jour. Biol. Chem. 37 : 465–496, illus.

(46) PETTENKOFER, M. V., and VOIT, C.
1869. UEBER DEN STOFFVERBRAUCH BEI EINEM LEUKAMISCHEN MANNE. Ztschr. Biol. 5 : 319–328.

(47) ROBSCHEIT-ROBBINS, F. S.
1929. THE REGENERATION OF HEMOGLOBIN AND ERYTHROCYTES. Physiol. Rev. 9 : 666–709.

(48) SHERMAN, H. C., and MUHLFELD, M.
1922. GROWTH AND REPRODUCTION UPON SIMPLIFIED FOOD SUPPLY. II. INFLUENCE OF FOOD UPON MOTHER AND YOUNG DURING THE LACTATION PERIOD. Jour. Biol. Chem. 53 : 41–47.

(49) SMYTHE, C. V., and MILLER, R. C.
1929. THE IRON CONTENT OF THE ALBINO RAT AT DIFFERENT STAGES OF THE LIFE CYCLE. Jour. Nutrition 1 : 209–216.

(50) TITUS, R. W., and HUGHES, J. S.
1929. THE NUTRITIONAL VALUE OF COPPER IN POWDERED WHOLE MILK. Jour. Dairy Sci. 12 : 90–93.

(51) WADDELL, J., ELVEHJEM, C. A., STEENBOCK, H., and HART, E. B.
1928. IRON IN NUTRITION. VI. IRON SALTS AND IRON-CONTAINING ASH EXTRACTS IN THE CORRECTION OF ANEMIA. Jour. Biol. Chem. 77 : 777–795, illus.

(52) WHIPPLE, G. H., and ROBSCHEIT-ROBBINS, F. S.
1926. BLOOD REGENERATION IN SEVERE ANEMIA. VII. INFLUENCE OF DAIRY PRODUCTS ON HEMOGLOBIN PRODUCTION. Amer. Jour. Physiol. 79 : 280–288.

(53) WILLIAMSON, C. S., and ETS, H. N.
1926. THE EFFECT OF AGE ON THE HEMOGLOBIN OF THE RAT. Amer. Jour Physiol. 77 : 480–482.

U. S. GOVERNMENT PRINTING OFFICE : 1933